CHARACTERIZATION OF A VIRUS CAUSING DISEASE IN SOLANUM AMOTAPENSE SVENSON

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

BOTANY

BY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1986

A B S T R A C T

A mosaic disease of Solanum amotapense was investigated and was found to be due to a virus which was called Solanum amotapense mosaic virus (SaMV). SaMV has a limited host range. Out of 80 plant species inoculated only 13 species were infected. SaMV evoked systemic symptoms in most of the solanaceous hosts except 4 viz. N. tabacum cv. White Burley, N. sylvestris, N. mistica and Datura stramonium on which necrotic local lesions were evoked. Necrotic local lesions were also evoked on C. amaranticolor, C. album and C. murale. SaMV is transmissible by manual sap inoculation and by grafting. No vector was found among the aphids, white flies and nematodes, tested.

SaMV has thermal inactivation point of 70°C; dilution end point of 1.06; and longevity in vitro of 16 days. SaMV attained maximum concentration 13 days after inoculation in N. tabacum cv. Anand-3.0.01M phosphate buffer pH 7.0 was found to be the most suitable medium to maintain the infectivity of the virus. SaMV was purified by a method involving extraction in 0.1M phosphate buffer pH 7.0 containing 0.01 per cent sodium sulphite, 1 per cent sodium chloride and 0.01M diethyl dithiocarbamate. Last traces of host proteins and other contaminating materials were removed by rate

zonal density gradient centrifugation. Purified preparations exhibited one light scattering band in sucrose density gradient columns. A single peak was observed in the analytical ultra centrifuge. The sedimentation coefficient was calculated to be 173S. Purified preparations gave an ultra-violet absorption spectrum typical of nucleo-proteins showing 5 per cent RNA. Purified preparations revealed the presence of rigid rods of 318 x 18 nm in the electron microscope. SaMV antiserum gave precipiten test titer of 120. SaMV showed serological relationship with cucumber green mottle mosaic, pumpkin mosaic, cowpea mosaic (a TMV Strain) and bottle gourd mosaic viruses. It seems that SaMV is a hitherto unreported strain of tomato mosaic virus.

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C O N T E N T

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This is to certify that the thesis entitled "Characterization of a virus causing disease in Solanum amotapense", submitted by Mr. Sajid Husain to the Aligarh Muslim University, Aligarh for the award of the degree of Doctor of Philosophy, is a faithful record of the bonafide research work carried out by him under my supervision and guidance.

PROF. KHALID RAHMAN
M.Sc., Ph.D.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Prof. Khalid Mahmood, Department of Botany, Aligarh Muslim University, Aligarh for his constant advice, guidance and encouragement during the course of investigation and writing of this manuscript.

I am indebted to Prof. S.K. Saxena, Chairman Department of Botany, Aligarh Muslim University, Aligarh for providing necessary laboratory facilities.

I am also thankful to Prof. M.D. Misra, Prof. (Miss) Atrey, Dr. F.R. Niazi, Dr. S.O.A. Naqvi, and Dr. S.M.P. Khurana for providing necessary facilities in their laboratories.

Sincere thanks are also due to Mrs. Shahnaz Fatima and Miss Khawaja Bano for their help and encouragement.

Husain

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CHAPTER 1

INTRODUCTION

Solanum amotapense Svenson, a member of the family Solanaceae, was first reported by Svenson (1946) from Peru who described the species as follows :

An erect bushy plant, leaves alternate, ovate-acuminate, 9 cm long 5 cm broad with prominent nerves, slightly hairy, petiole 2-4 cm long; inflorescence terminal receme, peduncle 4-5 cm long, calyx 3-4 mm long, 5 toothed strigose; corolla white, shortly cambanulate 1-1.5 cm in diameter, 5-lobes acute, pubescent on outside; stamens 5, filaments 5, equal in length, 05-1 mm long, anthers 5-6 mm long, 1.5-2.0 mm broad with porous dehiscence; style glabrous, 5.0 mm long; ferries globular, glabrous 1.0-1.5 cm in diameter; seeds deltoid 5 mm across, compressed, yellow.

The seeds of S. amotapense were acquired from Prof. J.G. Hawkes, Botany Department, University of Birmingham, U.K. for experimental purpose. Seedlings raised from these seeds were grown in experimental plots of the Department of Botany. Some of these plants exhibited green mosaic, particularly on the emerging leaves (Fig.1). The older leaves exhibited only mild symptoms. No symptoms were discernible on stems and flowers. Four out of six plants exhibited these symptoms.

The prevalence of a disease on an introduced plant attracted my attention. Attempts were made to characterize the causal agent of the disease. The present study deals with the characterization and identification of the causal agent based on host-range, symptomatology, transmission, bio-physical properties, purification, electromicroscopy and serology.

CHAPTER 2

REVIEW OF LITERATURE

The present review, in the absence of any report of a virus on S. amotapense, concerns with the viruses reported to occur on different genera of the family Solanaceae in general and different species of the genus Solanum in particular.

Quanjer (1922) reported four diseases of potato from Holland. Leaf roll was characterized by rolling and upward position of upper leaves, which showed reddish coloration. Phloem necrosis was also found in infected plants and the disease was transmitted by aphids. Mosaic was characterized by pale patches on the leaflets. Aphids carried this disease and it was also transmitted by grafting to tomato and tobacco but not by rubbing healthy plants with diseased sap. Aucuba mosaic was characterized by prominent localized yellow patches. It was transmitted by grafting to tobacco and potato plants. Datura stramonium, Solanum dulcamra, Nicotiana tabacum, Atropa belladona and Hyoscyamus niger were found symptomless carriers of the disease. Crinkle disease produced symptoms like those of mosaic but there were marked corrugations, the margins and tips curled downwards. The leaflets were brittle and turned yellow prematurely and the lower leaves dropped off. Necrotic

areas were found in the parenchyma, near the conductive tissue of the phloem. The disease was transmitted by grafting to potato, tomato Hyoscyamus niger, Datura & stramonium.and Atropa belladona. The author suggested proper seed selection as a method of control. Ducomet (1922) while studying the "degeneration" disease of the potato observed that varieties particularly subject to leaf roll are more likely to be attacked by mosaic than vice versa. None of the 70 varieties tested proved really immune. He concluded that the disease is not the result of cultivation since some wild species also became infected. Gardner and Kendrick (1922) studied over-wintering of tomato mosaic in Indiana and suggested that the virus might be carried over winter by means of hot house tomato crops in tomato seeds, in related perennial weed hosts or by insects. Perennial weeds were found to be important agents in the over-wintering of the disease. It was found in the weeds Physalis subglabrata, P. virginiana P. heterophylla, and Solanum carolinense and was transmitted from each of these to tomato. The disease was transmitted form tomato to the annual plants, Solanum nigrum, S. integrifolium and Lycopersicon pimpinellifolium but not to Datura stramonium. It was also transmitted from tobacco to tomato and on cultivated Physalis pubescens. Susceptible annual weeds were considered responsible in the spread of the disease during the growing season.

Eradication of solanaceous weeds, specially the perennial ones and those in and near tomato was recommended as a control measure against mosaic.

Overwintering tobacco mosaic disease was discussed in the 35th annual report of the Kentuky Agricultural Experimental Station (Anon, 1922). It was reported that the mosaic disease of tobacco was carried in the root stocks of the bull neetle (Solanum carolinense) and ground cherry (Physalis sp.), but mosaic diseases of bean, red clover, soybean and pokweed (Phytolacca decandra) were not transmitted as well as leaf roll of potato to tobacco.

Palm and Jochems (1924) tabulated some solanaceous and non-solanaceous plants as disease carriers for tobacco mosaic. They were Solanum ferox, S. Lycopersicum, S. melongena, S. verbastifolium, S. torvum, Citrullus vulgaris, Scoparia dulcis, Capsicum annuum, Physalis angulata and P. minima.

Johnson (1926) studied some differential hosts of tobacco mosaic virus (TMV) and reported Solanum rostratum a new host of TMV while wounded stems of Datura stramonium, potato, pepper and other plants reacted with the production of conspicuous lesions. Sometimes Nicotiana glutinosa and N. rustica were killed by stem lesions. N. glauca was stunted as a result of tobacco mosaic virus infection but with little or no mottling.

Solanum melongena (egg plant) was infected by the virus with difficulty and seldom showed mottling though stunting and stem or leaf necrosis were prominent in young plants.

Transmission of mosaic disease by aphid from tomato to potato and tomato in a limited number of cases under controlled conditions was reported in the thirty fifth annual report of Washington agricultural experimental station (Anon, 1926). No transmission of mosaic through the seeds of Solanum nigrum and Datura stramonium was obtained in repeated tests.

Van Der Meulen (1928) studied the intertransmissibility of the mosaic diseases of various agricultural crops. He concluded on the basis of cross-inoculation experiments that the mosaic diseases are very strictly specilised on their specific hosts. On the other hand white clover mosaic was transmitted to potato through Aphis rhamni but not through M. persicae. The mosaic occurring on Solanum nigrum was found distinct from that of the edible potato and the two were found intertransmissible by means of M. persicae.

Wingard (1928) investigated the occurrence and symptoms of tobacco ring spot on a number of species and varieties of Nicotiana and on a variety of hosts other than tobacco. Infection was obtained on 38 genera in 17 families by swabbing their

leaves with cotton plugs soaked in the fresh sap and macerated tissues of a Kentucky Yellow tobacco plant exhibiting typical symptoms of ring spot. The first symptoms of ring spot infection appeared 3 days after inoculation and became systemic 10 days later. In most of the cases, the infection was confined to the leaves but in several tobacco varieties stems were affected and in vegetable marrow fruits were affected.

Likhite (1929) transferred tobacco mosaic to tomato, Petunia hybrida, Solanum nigrum and Hyoscyamus niger by grafting and in these he observed the presence of crystals in the epidermal tissues and hairs.

Valleau and Johnson (1928) observed in the course of their work on the virus diseases of tobacco, that the incidence of disease in tobacco was always more frequent in or near fields in which potato has been grown for several years. He concluded from transmission experiments that the virus diseases originate largely from the weeds, the eradication of which was considered essential to the protection of healthy seed potato destined for certification.

Kotila (1930) studied the effect of roguing on the control of virus diseases of potato. The field was rogued at weekly intervals, beginning about six weeks after planting and was sprayed six times against aphids with Bordeaux mixture plus nicotine sulphate. After the first roguing, primary streak

(symptoms of which were identical with those of rugose mosaic) and primary leaf roll were the only disease found in the field. Tubers selected at random when indexed in the green-house showed a high percentage of a mosaic and leaf roll infection despite precautions taken in the matter of aphid control.

Smith (1931) while studying the virus diseases of potato investigated a ring spot disease of Solanum capsicastrum. He noted the great diversity of symptoms evoked by the virus on different hosts. Transmission of the virus using M. persicae was successful only with certain hosts. He concluded from his results that there is close parallelism in the behaviour of this ring spot virus to the viruses of the potato mosaic-crinkle streak group previously described by him and that it is a single entity rather than a complex of viruses.

Bald and Samuel (1931) reported the symptoms of the spotted wilt disease of tomatoes, a method of inoculation which was invariably successful, physical properties of the virus in plant sap and transmission of the disease by Frankliniella insularis. Spotted wilt was experimentally transmitted from tomatoes to tobacco and 14 other species of Nicotiana as well as to 7 species of Solanum, Capsicum annuum, Datura stramonium, Hyoscyamus niger, Lycium ferocissimum, Petunia hybrida, Physalis peruviana, Salpiglossis sp., Schizanthus sp., Aster sp. and Chrysanthemum sp.

Hoggan (1934) reported that tobacco mosaic virus was transmitted with some regularity from tomato and Lycopersicum pimpinellifolium but occassionally from 8 other hosts tested and no evidence of transmission was found with the remainder. Macrosiphum gei transmitted most from any host and Myzus persicae the least. Only two cases of transmission from tobacco to tobacco were obtained. In parallel tests high percentage of transmission of cucumber mosaic virus was obtained from all hosts tested.

Chamberlain (1934) investigated the appearance, cause and preventive treatment of tomato mosaic. He found that in small range field trials the average yield of diseased plants was reduced by 39 and 91.2 per cent by mild and severe mosaic, respectively. Both diseases were transmitted from tomato to tobacco, cape goose berry (Physalis peruviana) and black night shade (Solanum nigrum). He further found that healthy tomato plants growing alongside plants infected with mild or severe mosaic did not get infected although the aphids Macrosiphum gei and Myzus persicae were abundant on the healthy and diseased plants. He concluded that the spread of the disease by these insects is not extensive in the field and both mild and severe mosaic are mainly disseminated in New Zealand by pruning knives and the hands of the cultivators and that the virus may be removed from the hands by thorough washing with

soap and running water. Evidence obtained from glass house further indicated that the use of seed from infected plants is an important factor in perpetuating the disease from one season to the next. It was suggested that once mosaic has appeared in a crop, an interval of 14 days should be left between pruning so that all plants infected at one pruning show characteristic symptoms and may be removed before the next pruning.

Caldwell (1935) discovered a strain of yellow aucuba mosaic virus of tomatoes characterised by the production of a very faint mottle, typical of winter symptoms with little leaf distortion or stunting of the plant. It differed from the ordinary yellow mosaic in the symptoms evoked by it on Nicotiana glauca, N. glutinosa, tobacco, Solanum nodiflorum and Zinnia. This virus was considered a distinct strain and not an attenuated form of the other as its virulence was not enhanced by repeated passage through susceptible plants. The differences in symptoms were found suggestive of the existence of a hitherto unrecognised strain of the virus. It was shown that inoculation of healthy host plants with one of the strain completely immunizes them against infection with the other. Similar experiments were performed with other tobacco and tomato viruses and on the basis of the results four types of reactions were distinguished, (a) a virus may completely inhibit the development of another in the host tissues; (b) second virus may multiply in the tissues without inducing

typical disease symptoms; (c) The two viruses may multiply each inducing symptoms typical of its specific disease and (d) the effect of the second virus may be to intensify the severity of the disease symptoms induced by the single virus. It was believed that there must be either only a few loci in the tissues at which the virus can multiply or more probably there is type (a) reaction(some reaction of the plant to the first virus infection which prevents the multiplication of the second).

McClean (1935) described host range of the bunchy top virus of tomatoes in South Africa. Solanum aculeatissimum, S. aculeastrum, S. duplosinuatum, S. incanum, S. panduraeformae, S. nigrum, S. sodomaerum, Nicandra physaloides, Physalis anqulata, P. viscosa, tobacco, egg plant, cape goose berry (P. peruviana), Petunia, pepper (Capsicum annuum) and the potato were found hosts. Symptoms of bunchy top virus were marked in P. anqulata and S. nigrum. He suggested eradication of solanaceous weeds from the vicinity of tomato fields as important preventive measure.

Hoggan (1935) described two viruses of the cucumber mosaic group on tobacco. The first produced bright yellow mottling on cucumber, tomato, poke weed (Phytolacea decandra), spinach, night shade (Solanum nigrum) and egg plant. This was considered to be a variant of the ordinary cucumber mosaic

virus. The second produced a milder type of symptoms then the ordinary cucumber mosaic and also differed from the latter in physical properties in plant sap. This was termed cucumber mild mosaic virus.

Beale (1934) obtained positive precipitin reaction when extracts of Solanum melongena, S. sisymbrifolium, Physalis peruviana, Capsicum minimum and C. frutescens (C. annuum) affected with Johnson's tobacco mosaic virus, and Turkish tobacco affected with aucuba mosaic and attenuated tobacco mosaic were reacted with antiserum to tobacco virus I. Extracts from other solanaceous plants affected with mosaic diseases other than tobacco mosaic reacted negatively with the antiserum, She also suggested a method for determining the antigenic content of saline extracts of tobacco virus I and modification to Holmes local lesion method for the estimation of the active virus concentration in these extract.

Smith (1935) described symptoms incited by a virus from a tomato plant exhibiting typical symptoms caused by tomato streak virus No. 1 on tobacco (White Burley), Nicotiana glutinosa, N. langsdorffii, tomato, Datura stramonium, Hyoscyamus niger, Petunia spp. and potato (Arran victory). The isolate was considered to be green strain of the virus. This strain was maintained by transfer in White Burley tobacco and after four years one of the inoculated plants developed a small yellow spot. Inoculations were made from this spot into tobacco plants

and after 6 such transfer in which yellow tissue alone was used for inoculation a virus was isolated which evoked a bright yellow mottling on leaves of White Burley tobacco. The yellow strain could not be isolated absolutely free of the green strain. It was concluded that the green strain (which could be isolated absolutely free from the yellow) immunizes the tobacco plant against the yellow * strain. Transmission of tomato streak and tobacco mosaic to healthy susceptible plants was obtained by spraying them with a suspension of these viruses from an atomizer.

McClean (1935) in further investigation on the bunchy top disease of tomato reported its physical properties in plant sap and transmission to several hosts. Shepherd (1936) reported that tobacco mosaic prevalent in Black river district in Mauritius could be checked by systematic roguing late in the season.

Bald (1937) recovered a virus from Arran Crest or Arran Pilot potatoes which was carried without symptoms in a number of solanaceous hosts. The virus was repeatedly recovered over a period of one year from the tubers of the affected plants. The presence of virus failed to protect the plants from infection by y-type virus. Other hosts containing it were readily infected with X and X + B viruses. The virus was identical to potato virus I.

Clinch et al. (1936) compared aucuba mosaic of potato, potato tuber blotch virus and a latent virus from the Dutch potato Monocraat. All three were readilly transmissible by sap from potato and other solanaceous plants. They produced identical diagnostic symptoms on Solanum nodiflorum and Capsicum annuum, consisting of brown or purple bordered spots followed by rusty purple discoloration and mosaic. None of them produced any symptoms when inoculated to tobacco, Datura stramonium and Petunia. The physical properties of all the three viruses were found to be similar. The tuber blotch virus was transmitted by Myzus persicae but only in the presence of virus A. This and the Monocraat virus were considered to be identical. Tuber blotch virus was designated F with the Monocraat virus as a synonym and aucuba mosaic virus G.

Trotter (1936) observed ring spots on Cestrum paraqui plants which had shown mosaic symptoms in the previous year. Diseased plants of C. paraqui showed severe aphid infestation and those with the most conspicuous virus symptoms were growing near large areas sown to potato, tomato and tobacco.

Pole Evans (1937) reported that tomato and tobacco crops suffered extensively from mosaic in the Rustenberg area of Transval. The disease caused serious depriciation of quality as well as quantity in the foliage of affected plants. The

virus responsible for tomato bunchy top was found transmissible through the seed to seedlings of Solanum incanum as well as to Datura stramonium which showed no visible symptoms and acted as a carrier.

Alexandri (1937) detected egg plant mosaic in glass house plants in 1936. The virus caused 50 to 60 per cent damage earlier in the field. The virus was inactivated by exposure to a temperature of 60°C and to 64 per cent alcohol at 96° but not by dilution upto 1:1000. A number of solanaceous plants were infected experimentally.

Chamberlain (1937) reported high incidence of tobacco mosaic from Nelson District of New Zealand. Infection increased from under 10 per cent in 1933 to well over 25 per cent in 1937. It was found experimentally that when infection occurred shortly after the plants had been set out in the field it caused 44 and 78 per cent reduction of yield in a Virginian and a Burley variety, respectively. Leaf from the affected plants was useless. Several solanaceous hosts attacked locally, were reported. Healthy plants from which laterals were removed by men who had previously performed the the same operation on mosaic plants developed 83 per cent infection after 3 weeks. It was further suggested that the disease may be carried in or with the seed.

Pal and Tandon (1937) differentiated five types of leaf curl disease of tobacco in Northern Indiabased on symptomatology, reaction on hosts and transmission and designated them as A, B, C, D, and X. They recommended roguing of diseased plants following detection and their replacement by healthy ones.

Milbrath (1939) suggested that tomato tip blight virus can be differentiated on the basis of symptoms induced in tomato, Datura stramonium, Solanum capsicastrum, Bliss Triumph potato, nasturtium (Trapaeolum) and tobacco. Stem streaking and circular necrotic foliar lesions on tomato were considered outstanding features of infection either natural by thrips or artificial. Some important differences between the tomato tip blight and other tomato diseases were outlined.

Silberschmidt and Kramer (1938) collected mosaic diseased tobacco plants from various districts of Sao Paulo, Brazil and characterized them into six categories with respect to their symptoms. Symptoms induced in some wild solanaceous hosts after inoculation with Sao Paulo strain of tobacco mosaic virus have also been detailed. S. variable was found to be a carrier. Eradication of these hosts from the vicinity of tobacco plantations was suggested for controlling the disease.

Chamberlain (1939) reported that in green house inoculation experiments juice of diseased cucumber transmitted infection

to tobacco, Nicotiana rustica, Physalis peruviana, egg plant, Datura stramonium, chilli, Petunia hybrida, blue, yellow, and flowering lupins (Lupinus angustifolius, L. Luteus and L. polyphyllus), pansy, violet, (China) aster (Callistephus chinensis), Primula sinensis, P. obconica and Spinach.

Thomas (1940) distinguished five different strains of tobacco mosaic virus. Thirty six varieties of brinjal (e g plant) were tested under natural conditions. All were found susceptible to little leaf disease which w s transmitted by grafting to tomato, tobacco, Datura fastuosa, Solanum xantho-carpum, S. torvum, S. trilobatum and a wild var. of egg plant. Jassid, Eutettix phycitis transmitted the virus successfully whereas Empoasca devastans was less consistent.

Costa, Lima and Forster (1940) studied a virus disease of tobacco which caused white necrosis as early and "Cabbage tobacco" as late symptoms. The disease affected Virgina Bright, Samsun, Turkish Kentucky, Clor Branca, Gendertheimer, and other tobacco varieties, as well as Nicotiana sylvestris in several districts of Sao Paulo, Brazil. Symptoms developed in three successive stages, of which the first two i.e., white necrosis and apparent recovery corresponded with Johnsons streak while the third the cabbage leaved phase appeared to be new. The

disease was transmitted by rubbing to Nicotiana glutinosa, N. rustica, N. alata, N. repanda, N. Langsdorffii. N. longiflora, S. nodiflorum, Nicandra physaloides. The symptoms evoked on Nicotiana sylvestris and N. repanda resembled those observed on tobacco. The other hosts developed foliar habit and partial separation of the petals. The thermal in-activation point of the virus was between 50°C, longevity in vitro 12 to 24 h, and tolerance to dilution 1:100. The physical properties resembled those of tobacco streak virus.

Magee (1940) studied transmission of infectious clorosis of banana and obtained transmission with Macrosiphum gei, Aphis gossypii and an unidentified aphid. The virus was transmitted using Aphis gossypii from cavendish banana to Musa ensete and to a seeded Musa of unidentified species. It was transmitted from the last named by A. gossypii to cavendish and Gross Michel banana, abaca, Canna indica, cucumber, squash and tomato. It was readily transmitted by mechanical inoculation from cucumber and squash to cucumber, squash and tobacco and from tobacco to cucumber, squash and the seeded banana. Symptoms evoked in tobacco, cucumber and squash were characteristic of cucumis virus 1. Fern-leaf symptoms developed in tomato when the virus was transmitted by A. gossypii. It was concluded that the virus is in fact cucumber virus 1.

Hill (1940) observed big bud of tomato on Solanum nigrum and Datura stramonium. Such symptoms were also observed on egg plant and were similar to those obtained by grafting infected tomato scions on healthy egg plants. Occurrence of such symptoms on non-solanaceous hosts indicated a wide host range. It was considered that the disease known as big bud in Australia, stolbur and Montar of tobacco, tomato, and chilli in U.S.S.R., and little leaf of egg plant in South India were either due to the same virus or very closely relative viruses.

Bremer (1941) tabulated some hosts of tomato leaf-roll in Germany and differentiated on the basis of symptoms, three forms viz. basal, total and apical. Other members of the family Solanaceae suffering from the disease included potato, Solanum nigrum, & S. pruniformae and Lycopersicon humboldtii.

Samsan & Imle (1942) observed a ring type of virus disease of tomato in nine varieties of tomato including Greater Baltimore, Marglobe and Pritchard. Virus from tomato and Datura stramonium was successfully inoculated to other tomato vars. and 19 members of the family Solanaceae. Symptoms of the ring spot resembled those of spotted wilt and the host range was narrower. Thermal death point was higher (56° to 58° compared with 42°C) ageing in vitro was 21 h compared with 6 for spotted wilt. Furthermore, plants infected with ring spot were not protected by suspected

spotted wilt virus. The host range was narrower than that of spotted wilt. On the basis of differences in symptoms, host range and properties, it was concluded that the tomato ring spot is distinct from the tobacco ring spot, potato ring spot, and tobacco ring mosaic viruses.

Kohler (1943) carried out experiments on the transmission of K-virus of potato using tubers of infected plants as source of virus. Typical symptoms developed in inoculated plants together with symptoms of x-virus which was considered to be latent in the variety. It was further established by aphid aransmission that the leaf-rolling mosaic of the small leaved wohltman variety is caused by virus K. Varieties parnassia and wohltman were infected by sap inoculation.

Smith & Markham (1944) described two new viruses affecting tobacco and Arabis hirsuta which appeared on plants growing in an insect proof glass house. Both viruses induced ringspot symptoms on tobacco but that from A. hirsuta was distinguished by characteristic curling and shredding of the central leaves of diseased plants. This virus was designated as Arabis mosaic and considered distinct from cabbage or cucumber mosaic. It was inactivated by a 10 min exposure to 60°C and by dilution to 1:1000. Its longevity in vitro ranged from 38 to 72 h. The virus was sap transmissible. Tobacco, Nicotiana glutinosa,

Solanum nodiflorum, cucumber, and Canadian Wonder French beans were susceptible to the virus. The virus from tobacco was designated tobacco broken ring spot virus because of incomplete ring formation, resembled Arabis mosaic virus in its thermal inactivation and dilution end point but its longevity in vitro was longer (6 days at room temp. and was readily sap transmissible. White Burley and Kawala Turkish tobacco, N. glutinosa, French bean and cucumber were found susceptible showing broken ring spots. Frehch bean and cucumber were considered to be of diagnostic value in differentiation of the two viruses.

Delle Coste and Zabala (1946) summarized results on tomato "black pest" and tobacco corcovo (two distinct manifestation of tomato spotted wilt virus). Thirty one species were found hosts of the virus by sap inoculation. In all cases positive results were obtained when sap of the inoculated plant was tested by inoculation to N. glutinosa and tomato. Noordam (1943) called attention to corroborative work done in Holland on tomato spotted wilt virus and confiermed the presence of the virus in Holland.

Clinch (1944) observed a severe disease in a single potato plant of a small crop of British queen potatoes grown from

certified seed in Dublin in 1934 and subsequently detected it in 1938 in seed potato stocks and in the field was shown by inoculation experiments to be caused by potato virus X only. The symptoms, therefore, considered to be due to a virulent strain of virus X were described in 32 potato varieties, in all of which except three where top necrosis occurred, a primary stage of leaf necrosis and defoliation succeeded in the same year by severe mosaic and stunting. Solananceous hosts shown to be susceptible were Datura stramonium, Nicotiana glutinosa tomato, Solanum nodiflorum, S. nigrum, chilli and White Burley and Orinoco tobacco. Only Lamium hybridum reacted systemically out of a large number of non-solanaceous hosts tested. Local lesions were induced in Veronica agrestris, beet root and marigold. The physical poroperties and serological reactions of the severe strain were similar to those of other X strains. Plants carrying mild strain could not be infected by severe strain by sap inoculation but were infected by grafting.

Vasudeva and Samraj (1947) reported that Sutton's Early Market tomato plants developed a faint mottle when inoculated in the glass house with the sap of tomato plants affected by "Smalling disease". Plant appeared normal after the disappearance of the symptoms byt still carried the virus. The infected tomato plants developed intracellular inoclusions in the form of

x-bodies and cubical crystals which were more prominent in the epidermal hairs. The X-bodies were confined to the two lowest cells, one in each cell, lying in close proximity to the nucleus which was enlarged and slightly deformed. The X-bodies were usually circular and averaged 25 μ in diameter. The number and size of the crystals varied in different cells, the lager ones measuring about 6 μ. White Burley, Harrison's Special and German Samsun tobacco when inoculated with the symptomless virus reacted by a pale green mottling spreading from the tip and margin over the netire surface of the inoculated leaves and ultimately involving all the others. The symptoms disappeared as in tomato, leaving the plant apparantly normal. The only other plant to evoke faint mottle was Solanum nigrum. The virus was inactivated by ten minute's exposure to 59°X and was still infective in extracted sap after 24 h at room temperature.

Vasudeva and Lal (1945) isolated Solanum virus 2 (Potato virus Y) From Phulwa potato plants showing either negligible mosaic and veinal necrosis or severe mosaic only. The vars. Gola magestic, President Windsor Castle and Talisman (upto-date) exhibiting the same symptoms followed by acropetal necrosis and all the phulwa plants except a few showing negligible mosaic and veinal necrosis gave a mixture of potato virus Y and Solanum virus I (Potato virus X). Potato virus Y

was isolated by passage of the complex through Petunia hybrida. The virus was inactivated after exposure to 54°C and by dilution of 1:1000 and by storage in extract for 24 h.

Stubb (1947) transmitted by sap inoculation a destructive wilt disease of broad bean to healthy broad bean plants. The array of symptoms produced on broad bean in the field was described. Varieties Leviathan long pod, Cole's dwarf profilic and tick beans were found equally susceptible. Pea vetch, Tangier pea (Lathyrus tingitanus), sweet pea, blue lupin (Lupinus angustifolius), Datura stramonium, D. ferox, D. metal, Solanum nigrum, tomato, Petunia hybrida, Chilli, tobacco, Nicotiana glutinosa, beet var. Detroit, Chenopodium album, Spinach, and Antirrhinum majus all reacted to inoculation with systemic symptoms except beet which showed only chlorotic spots. The virus was inactivated between 58-60°C and remained infective at 21°C for 54 but not 72 h and could withstand dilution upto 1 in 10,000. Difficulty was encountered in transferring the virus from plants long infected. Removal of infected plants and delaying sowing as long as possible was recommended for controlling the disease.

Chamberlain (1947) studied the tomato streak which produced severe streaking of tomato leaves, stems and fruits and a less obvious mosaic mottling of the foliage. Tobacco, chillies,

pepper, egg plant, *Physalis peruviana* and *Solanum nigrum* were also infected.

Kohler and Panjan (1944) isolated from a mosaic-diseased tobacco plants a virus which they designated as paramosaic virus. This virus differed widely in its symptomatology from the tobacco mosaic virus but was similar to TMV in physical properties in plant sap. White Burley tobacco plants inoculated with TMV green strain contracted no further infection when super-inoculated with para tobacco mosaic virus. However, when Samsun plant pre-infected with para tobacco mosaic virus were super inoculated with aucuba variant of TMV, the latter made unimpeded progress. It was concluded that PTM is an abnormal variant of TMV, much more stable than the latter.

Vasudeva, Garg and Azad (1949) reported that when tomato plants were inoculated with sap from potato plants showing severe crinkling and mottling, veinal necrosis and necrotic spot consistently developed. The disease was transmitted by mechnical inoculation from tomato to tobacco, *Datura stramonium*, *Solanum nodiflorum* and *Petunia hybrida*. On the basis of reaction on these differential hosts, it was concluded that the necrosis was caused by potato virus X and Y. Kohler (1951) suggested that potato virus A, X and Y and tobacco ring spot virus can be differentiated by inoculating potato juice samples simultaneously into young plants of Samsun tobacco and *Datura stramonium* or *Gomphrena globosa* as well as into *S. demissum*

leaves.

Vasudeva and Nariani (1952) described the host range of bottle gourd mosaic virus. An isolate of the virus from Lagenaria leucantha caused localized infection without inducing symptoms on inoculated leaves of tobacco, White Burley, Solanum nigrum and S. nodiflorum but did not infect tomato or chilli. The activity of the virus was inhibited by leaf extract of D. stramonium.

Klesser (1951) noticed a new virus disease on two sets of red currant plants imported from Holland into England. The plants had malformed dark green leaves with vivid yellow rings. The virus was inactivated at 66° to 68°C tolerated a dilution of 1:2000 but not 1:5000, lost infectivity after 7-9 days in vitro and was transmitted by sap only. The virus infected Nicotiana glutinosa, N. rustica, N. glauca, N. sylvestris, Petunia hybrida, cucumber, Amaranthus tricolor, Datura stramonium, Nicandra physaloides, Physalis angulata, Solanum muricatum, S. nigrum, S. nodiflorum, Spinach, Tetragonia expansa and Zinnia elegans. Infection protected the plants against tobacco ring spot virus.

Sprau (1951) reported the occurrence of a new virus disease-dwarf shrub virosis in potato. The disease was sucessfully transmitted to Ackersegen and other potato varieties,

tomato, Solanum demissum, S. nigrum, tobacco, Datura stramonium and Petunia by grafting but not by sap or insects.

Pushkarnath (1952) isolated a virus resembling potato virus Y in many respects, from Solanum jasminoides but the virus was not transmitted by sap inoculation or grafting to S. nodiflorum or potato (var. Craigs, Defiance, Arran Victory, and Majestic). Chilli and Datura tatula were immune. On Solanum jasminoides it evoked fleeting type of mottle while on White Burley tobacco, S. nigrum, Hyoscyamus niger, Nicotiana glutinosa Petunia and tomato to which it could be transmitted by sap inoculation a variety of symptoms developed. Only mottling symptoms developed on the last three. The dilution end point of the virus varied from 1:1900 to 1:2000; it retained infectivity for 24 to 48 h and was inactivated at at 60°C. Potato virus Y gave protection against S. jasminoides virus in cross immunity tests.

Volk and Bode (1952) investigated the host range of potato leaf roll virus. He successfully transmitted the virus to various hosts i.e., Hyoscyamus niger, Nicandra physaloides, Nicotiana rustica, Solanum demissum and S. acule by aphid Myzus persicae. Kohler (1952) studied ring spot or "bouguet" a virus disease of potato. He presented evidence on the

symptoms produced on beans (Phaseolus vulgaris), cucumber, Calendula, Solanum polycanthos, S. luteum, Datura gigantea and Nicandra physaloides by inoculation with two strains of the virus designated N and C, the former isolated from a typically diseased field plant of the Bone variety and the latter (the more virulent of the two) from Samsun tobacco inoculated with sap from a green house "eye cutting" of the cender tomato.

Sakimura (1953) observed mixed natural infection of potato virus Y and tomato spotted wilt virus in field plantings of tomato in Hawaii in 1950 and 1951. Virus Y was isolated after sub-inoculation on Nicotiana glutinosa and had a thermal inactivation point between 53° and 86°C, longevity in vitro from 48 to 72 h at 25 to 29° and from 66 to 82 days at 0 - 3°C, dilution end point was 1 in 300 and 1 in 1000. The virus was transmitted to egg plant, Solanum nodiflorum, Nicotiana glutinosa, N. rustica, tobacco Physalis peruviana, chilli (Capsicum frutescens) Petunia, Nicandra physaloides and tomato. Myzus persicae transmitted the virus to N. rustica after 1 to 2 day acquisition and 3 to 4 day infection feeding of 5-15 apterous young aphids per plant.

Anderson (1954) reported that aster ring spot virus first observed on California Wonder Chilli (Capsicum frutescens) in

1950 recurred in the two following seasons. It was experimentally transmitted to 15 host plants which included White Burley tobacco, Nicotiana glutinosa, cucumber, Trapeolum majus, tomato, Zinnia and cowpea. The virus was similar to cucumber mosaic virus in certain respects but differed from it in inducing no symptoms in cucumber, in the failure of aphid transmission and in the absence of serological relationship.

Greenleaf (1953) showed that a destructive wilt of Tobacco Chilli pepper (Capsicum frutescens) was caused by tobacco etch virus. The first visible symptoms were vein clearing and faint yellow flecks in the young leaves which appeared between 4 and 21 days after inoculation and were invariably accompanied by or followed by wilting. Five varieties of Capsicum pendulum including the commercial Serrano and Solanum pseudocapsicum were tolerant of the virus and S. carolinense developed only mild infection.

Cockerham and McChee (1953) designated the virus causing stunt disease of potato as potato dwarf virus. The virus was transmitted by sap inoculation to Nicotiana rustica and Essex wonder tomato which were symptomless and to Solanum demissum and S. nodiflorum which showed stunting, general chlorosis and distortion. Capsicum frutescens, Physalis floridana and White Burley tobacco became chlorotic, distorted and stunted.

Silberschmidt, Rostom and Maltos Ulson (1954) observed that sap from a Solanum sp. with crinkled leaves induced round yellow chlorotic spots on younger leaves of white Burley tobacco accompanied by stunting of the plant and vein banding of the younger leaves. Datura stramonium showed no symptoms. Thermal inactivation point was between 55° and 60°C. Myzus persicae after an acquisition feeding of 5 min produced round yellow spot in tobacco plants to which they were transferred. The causal virus was supposed to be a strain of potato virus Y.

Klinkowski (1954) reported that tobacco mosaic virus is inactivated by various products of fungal metabolism. The number of local lesions on Nicotiana glutinosa leaves were reduced from 67 to nil when the inoculum was mixed in equal parts with 3 weeks old Czapek-Dox culture filterate of Botrytis cinerea from primula and Cineraria, to 0.8 by the same species from Geranium and 1, 5, and 4, respectively by three strains of Rhizoctonia solani.

Chambers and Fisken (1954) reported that when infected raspberry leaves were ground with nicotine sulphate, the extract, after dialysis, contained viruses that produced ring spot symptoms on tobacco, cucumber, Petunia and other

solanaceous hosts. Six out of 400 Norfolk Giant raspberry seedlings inoculated with sap from a tobacco culture of raspberry leaf curl, developed typical leaf curl. This was the first demonstration of sap transmission of a virus affecting raspberry. These ring spot viruses were readily transmitted from infected tobacco and Petunia to Nicotiana rustica, N. glutinosa, Physalis spp. Tropaeolum and tomato. They produced local lesions on Phaseolus vulgaris, Datura stramonium and Hyoscyamus niger, from all of which virus was transmitted to Petunia. None of the local lesion isolates gave protection against infection by tobacco ring spot, Trapaeolum ring spot or tomato black ring viruses.

In a report of the Division of Mycology and Plant Pathology of the Agricultural Research Institute, New Delhi (Anon. 1954) cucumis virus 2C(cucumber green mottle mosaic virus) was transferred to Datura successfully. Cucumis mucronata and Phaseolus vulgaris developed typical mosaic symptoms on inoculation whereas the virus was carried without symptoms in tobacco at low temperature producing faint mottling at higher temperature. Local lesions were produced on Datura stramonium, Nicotiana glutinosa, and cowpea, while tomato, Solanum nigrum, and S. nodiflorum were found

symptomless carriers. During the testing of symptomless potatoes, a virus was isolated which ppoduced moraic symptoms on N. glutinosa, necrosis on chilli and transient mottling on tobacco. The thermal inactivation point lay between 55° and 60°C and infectivity was retained in expressed sap for 15 days at 6° to 10°C.

Munro (1955) studied the reaction of various solanaceous hosts to eight strains of potato virus Y is a search for differentials for strains not clearly distinguished on potato. The viruses were maintained on White Burley tobacco and sap inoculated. He found that the strain Y3 was unable to infect Physalis floridana while other strains produced necrotic lesions; those causing mild symptoms in potato inducing severeones in P. floridana and vice versa. Four of the test strains caused an acropetal necrosis in Nicotiana rustica, similar to the leaf drop streak characteristic of many strains of virus Y in potato. Y3 strain produced very severe necrosis and distortion in N. sanderae, while with other 4 strains there was only a transient vein clearing followed by vein banding. On White Burley the only distinctive strain reaction was that of tobacco veinal necrosis virus; Y2, Y4 and Y6 partially protected the plants from infection by this strain. It was concluded that a wider range of indicator plants

bring out the differences between strains of a virus more clearly.

Roland (1954) reported that potato virus F is identifiable by sap inoculation on the detached leaves of Solanum demissum. A method for serological detection of potato virus S was described. Out of 37 species belonging to Solanaceae tested for reaction to virus S, twenty six were susceptible namely Datura metal, tomato, Nicotiana rustica, S. acule, S. andigenum, S. chacoense, S. caldasii, S. commersonii, the 'S' var. of S. demissum, S. goniocalyse, S. longipedicellatum, S. polyandenium, S. pseudo-capsicum, S. subandigenum and twelve other spp. The virus was present in all the 40 commercial potato varieties tested.

Kohler (1957) reported thermal inactivation point of virus K (Potato leaf rolling mosaic virus) at 73°C. Munro (1959) suggested diagnosis of potato virus F (Potato aucuba mosaic virus) by means of Solanum miniatum. Many species did not evoke symptoms and others behaved erractically but S. miniatum proved a reliable indicator unaffected by changing environmental conditions. The symptoms grey rings, blotches, brown necrotic blotches, followed by chlorosis and leaf drop were more severe and distinct from those caused by potato virus A, X and Y.

Azad and Seghal (1958) isolated a virus occuring in a complex with tobacco mosaic virus by passage through Nicotiana glutinosa. The host range of the virus was restricted to family Sobanaceae. It caused severe systemic infection in tobacco and N. glutinosa and was transmissible to seven other Nicotiana spp. but not to N. glauca, potato or Capsicum, Datura metel and Nierembergia frutescens were symptomless carriers. In tobacco it caused vein clearing of young leaves followed by severe mottle, green vein banding, deep green blisters, malformation and downward curling of the leaves. The dilution end point was between 1:80 and 1:150 and thermal inactivation point between 72° and 78°C. Its longevity in vitro was about 9 days. Distortion mosaic of tobacco was suggested as the name of the virus.

Vasudeva (1955) reported that bringal (egg plant) mosaic virus could not be transmitted to Hyoscyamus niger, Papaver, Datura stramonium or Solanum torvum and was not seed borne. Only Vinca rosea out of 11 plant species proved susceptible to egg plant little leaf virus. Eutettix phycilis retained the virus through out its life.

Bagnall (1960) recovered a virus from symptomless Albion potato plants (a Dutch variety) and from F 451 seedlings

with severe necrosis. It proved to be potato virus F (potato aucuba mosaic virus strain). Thirty one species belonging to family Solanaceae became infected, except Solanum capsicastrum, S. miniatum, reacted only mildly and no better indicator than Capsicum annuum was found. Neither S. miniatum nor C. annuum were, however, reliable when potato virus X was present.

Anderson (1958) suggested the use of two cowpea strains of cucumber mosaic virus to identify other strains immunologically with tobasco pepper as the immunized plant. Graft inoculation showed that virus or virus like disease unrelated to the Capsicum virus diseases occur in S. carolinense which may also be infected by Capsicum viruses in the field.

Clark (1962) found Solanum demissum, S. raphanifolium, S. lanciformae, S. parodi and some lines of S. phureja resistant to potato leaf roll virus using viruliferous Myzus persicae. Bagnall (1963) isolated a virus from Solanum phureja, called SB 29 which resembled potato virus A and Y in particle size, vector relationship, and host range, but differed in host reaction and serology.

Gibbs et al. (1965) isolated a virus related to Ononis yellow mosaic virus from symptomless plants of Solanum tuberosum group from Colombia, Bolivia and Peru and named it Andean potato latent virus. The virus was sap transmitted to other

solanaceous plants and to Chenopodium amaranticolor and C. quinoa. It produced vein clearing, flecking and mosaic on Nicotiana glutinosa. Another virus also related to Ononis yellow mosaic named dulcamra mottle was isolated from S. dulcamra. Infected plants exhibited mottling and leaf puckering. Polyhedral particles from plants with dulcamra mottle virus or Andrean latent virus were similar to those of Ononis yellow mosaic and the viruses were related serologically.

Fedotina (1964) detected particles of TMV and inclusions in xylem vessels of tobacco and Petunia leaves and also in the leaves and flowers of Solanum luteum on staining with osmic acid and 2 and 5% trichloro acetic acid. The virus was present in 40 sections out of 95 examined.

Bode and Vogel (1965) outline a schedule for detection and separation of tobacco mosaic, tobacco rattle, potato X, potato Y, tobacco ringspot, lucerne mosaic, and cucumber mosaic viruses, by means of tobacco, Nicotiana glutinosa, Datura stramonium, cucumber, Chenopodium quinoa, Solanum demissum, Gomphrena globosa, D. metel and Phaseolus vulgaris.

Agur (1966) obtained a mutant NR, during studies on potato virus N, which incited yellows type symptoms on Nicotiana rustica. Leaves turned eyellow, terminal shoots

were necrotic and flowers on lateral shoots changed colour and structure. The mutant retained infectivity at a higher dilution (10^{-5}) in expressed sap for a longer period 48 h in vitro and had a higher thermal inactivation point than the original virus. Symptoms incited by NR mutant on tobacco, N. glutinosa, Solanum demissum, S. acaule and Nicandra physaloides were different from those of virus N.

Gaspar and Gyula (1970) investigated resistance of some Solanum species and their hybrid to potato virus Y. He found that in the progeny of S. andigenum, S. chacoense, S. commersonii, S. stoloniferum or S. demissum, potato virus Y could not be demonstrated after mechanical inoculation. These were considered as resistant, although type of resistant was not determined. Of 30 clones of various interspecific crosses 14 remained symptom free after inoculation; in nine no latent infection was indicated by the A6 test.

Krylov (1969) identified potato virus A, M, S and F by indicator plants. He recommended Lycopersicon pimpinellifolium and hybrid A-6 as indicator plants for A, Solanum rostrum for S., Nicotiana debneyi, Gomphrena globosa and Vigna sinensis for M and N. glutinosa, Capsicum annuum and S. miniatum for F.

Maduewesi (1967) transmitted a virus or viruses inducing mosaic pepper from Capsicum annuum to Solanum nigrum, N. glutinosa and C. annuum. Inactivation occurred after 4 days at room temperature and after 10 min at 64°C. No evidence of seed transmission was obtained.

Horvath (1969) differentiated viruses pathogenic to tobacco. He suggested that mechanically transmitted viruses viz. tobacco mosaic, potato A, X and Y(PVA, PVX, PVY), cucumber mosaic (CMV), alfalfa mosaic (AMV) and tobacco ring spot could be differentiated by the systemic and/or local susceptibility or immunity if Chenopodium amaranticolor, Crambe abyssinica, cucumber, Datura metel, D. stramonium, Lycopersicon pimpinellifolium, Nicotiana glutinosa, bean (Phaseolus vulgaris) and tobacco vars. Hicks Fixed A2-246 and Samsun. The stylet borne viruses (AMV, CMV, PVA, PVY) could be separated from the others by transmission by Myzus persicae. These could be differentiated from one another on Solanum demissum A6 hybrid, C. amaranticolor and L. pimpinellifolium.

Lockhart and Fischer (1974) identified and characterized a strain of tobacco mosaic virus causing severe losses in commercial crops of tomato in Morocco. Wild infected Nicotiana glauca and Solanum sodomaerum appeared to be the source of

the virus but means of spreading to tomato fields could not be determined. The virus was transmitted with difficulty from infected to healthy tomato by Myzus persicae but no transmission was obtained from N. glauca or S. Sodomaeum.

Schmelzer and Spaar (1975) investigated the reaction of cultivated potato vars. and wild potato spp. to cucumber mosaic virus. He reported that the local lesions readily developed by inoculation on all 20 vars. but one grown commercially in GDR. Partial systemic infection was exceptional. Two isolates of the virus on inoculation of 200 wild potato sources at the seeding stage caused systemic infection in all 10 series. Solanum acaula and S. demissum were particularly susceptible. The normal strain induced more systemic infection than the white strain. He concluded that European breeding material for cultivated potatoes appeared only slightly susceptible to the virus but resistance may be reduced in crosses with wild species.

Anjaneyulu and RamKrishan (1973) compared 22 isolates of tomato big bud virus from different natural hosts on egg plant under similar conditions. The isolate from Solanum sisymbrifolium induced prominent vein swelling containing abnormal phloem tissue on the leaves of egg plant; milder symptoms were induced on tomato, tobacco, Nicotiana glutinosa,

Datura fastuosa and D. metel. S. integrifolium, S. gilo and S. muccagnianum, resistent to the egg plant isolate, were susceptible to the other. In cross protection tests, inoculation by one isolate protected against infection by the other. Based on these findings the 2 isolates were considered to be 2 different related strains. The one from egg plant was designated as the 'severe' strain and the other from S. sisymbrifolium as the 'mild' strain.

Sastry et al. (1975) studied a mosaic disease of Solanum khasianum. The virus was mechanically transmissible to S. khasianum, S. sisymbrifolium, S. nigrum, Physalis floridana, White Burley, Harrison's special and xanthinc tobacco, Nicotiana glutinosa, N. rustica, Gomphrena globosa, Nicandra physaloides and Lycopersicon through seeds. The virus in crude sap was inactivated between 45 and 50°C; dilution end point was between 1:1000 and 1:10000, Infectivity was lost after storage for 24 hr at room ptemerature. The virus remained active upto 30 days in dessiccated leaves. This was considered to be a new virus.

Davis and Allen (1975) determined weed hosts of tobacco rattle virus in Idaho. They made inoculations from 27 plant spp. and TRV was recovered from the roots of Solanum nigrum, Brassica Campestris and Erodium cicutarium. S. nigrum was

found to be the most consistent source of TRV and caused the severest symptoms on Samsun NN tobacco. S. nigrum besides being an important wild host could be used as a bait for detecting tobacco rattle virus in field soils.

Chagas et al. (1977) isolated and identified potato virus Y from Solanum atropurpureum. The plants exhibited vein banding, chlorotic spotting and leaf curl symptoms. The virus was identified by means of test plants, physical properties in sap, morphology and reaction against PVY antiserum. The occurrence of natural infection in this wild plant indicated that it may be an important reservoir of the virus.

Vicente et al. (1979) isolated a virus from Solanum ciliatum and S. viarum with vein banding and leaf deformation and S. robustum with slight mottle symptoms growing in Sao Paulo region, Brazil. These plants were naturally infected and the virus resembled potato virus Y in its symptomatology on indicator plants, physical properties and morphology. It was suggested that these species may be reservoir of PVY for cultivated plants.

Chagas et al. (1978) investigated wild Solanaceae as experimental hosts of viruses. Solanum ciliatum, S. palinacanthum and S. viarum were inoculated with potato virus Y

and its necrotic strains PVY^N, potato virus X, tobacco rattle virus (TRV), tobacco mosaic virus, cucumber mosaic virus (CMV) and tobacco spotted wilt virus. In most cases systemic symptoms were produced. TRV and CMV did not infect S. palinacanthum or S. viarum.

Barradas et al. (1979) investigated some Solanaceae viz. Solanum lycocarpum, S. mamosum and S. robustum as experimental hosts of viruses. These species were inoculated with cucumber mosaic virus, potato virus X and potato virus Y necrotic strain. In most cases systemic symptoms appeared. Chracteristic symptoms of TRV and TSWV appeared on S. lycocarpum and of PVX, PVY, CMV and TSWV on S. robustum. S. lycocarpum was a latent host of TMV and S. mammosum of PVY^N. They concluded that all 3 spp. may be potential resevoir of the viruses to which they are experimentally susceptible.

Wilson et al. (1981) reported Solanum nigrum to be a new host of tomato yellow leaf curl virus. S. nigrum mechanically inoculated with tomato yellow leaf curl virus developed yellow vein mosaic on the leaves. Naturally infected. S. nigrum plants were observed in and around tomato (Lycopersicum esculentum) fields in Iraq. Bemisia tabaci transmitted the virus from infected tomato plant to S. nigrum.

Alexandre and Barradas (1982) reported Solanum mammosum, a valuable differential host for potato virus Y and potato virus Y strain N. S. mammosum proved to a ~ latent host for PVY^N and reacted with systemic chlorotic spots when inoculated with PVY. It was suggested that this species can be used as differential host for PVY and PVY^N.

CHAPTER 3

MATERIALS AND METHODS

Cultivation of plants : Plants were grown in clay pots of 4" or 6" diameter. The pots were sterilized by rinsing them with 4% formaldehyde solution 24 h before use. Pots were filled with a mixture of sand, soil and compost in ratio of 1:2:1 and sterilized for 2 h at 20 lb pressure in an autoclave.

Seedlings were raised in pots of 10" diameter and transplanted singly in smaller pots. Those which are not transplanted e.g. members of Cucurbitaceae and Leguminosae were raised directly in pots.

Virus culture : The isolate obtained from an infected Solanum amotapense plant growing in an experimental plot was used in this study. The infected plant exhibited mosaic symptoms. The culture of the virus was maintained on N. tabacum var. Anand-3 by periodic mechanical sap inoculation. To ensure identity of the virus periodic checks were made by inoculating various members of Solanaceae and Chenopodium amaranticolor.

Inoculations were made using the forefinger and employing carborundum 500 mesh, as an abrasive. Leaves were

rinsed by a gentle stream of water soon after inoculation. Standard extract was used as inoculum for maintaining the culture of the virus.

Standard extract : Standard extract was prepared from N. tabacum cv. Anand-3, inoculated 14 days earlier, Young infected leaves were taken, washed, and macerated in a mortar with pestle after addition of 0.01M phosphate buffer pH 7.0 and 0.01 per cent sodium sulphite in a ratio of 1:1 for each g of plant tissue. The macerate was passed through double layered cheese cloth. The extract thus obtained was refferred to as standard extract and used routinely as virus inoculum.

Virus assay : C. amaranticolor plants of same age and height having 6-8 leaves grown in identical conditions, were used. Lateral branches if any and unwanted leaves were removed. Leaves were dusted with carborundum 500 mesh before inoculation and inoculation was made using the forefinger. Leaves were rinsed immediately after inoculation by a gentle stream of water. Local lesions were counted 5 days after inoculation. Plants were arranged in a latin square and number of lesions per set tested are given at appropriate places in the text.

Host range & Symptomatology : Several species and cultivars of plants belonging to different families were inoculated with the standard extract. Inoculations were made manually using the forefinger and carborundum 500 mesh, as an abrasive. Plants of family Cucurbitaceae were inoculated at cotyledonary stage. Inoculated leaves of all plants were rinsed after inoculation by a gentle stream of water. At least 3 plants of each species or cultivar were inoculated and an equal number of the same were kept as control. All plants were kept in uniform light and humidity in an insect proof glasshouse. Each experiment was repeated thrice in different seasons and plants were observed upto 4 weeks after inoculation.

Transmission : Transmission studies were carried out using aphid, white fly and nematode as vectors. Transmission through seed of infected plant, soil and graft was also studied.

Aphid : Rearing of aphids : Virus free aphids were reared on appropriate host-plant. Five plants were covered by an aphid proof cage and placed on a zinc tray which was filled upto the brim by sand. The cage was made of a wooden frame having the top and two sides covered with glass and the other by wire gauze. New colony of each aphid species was maintained on respective host plant. The colony was reared under continuous light at about 22°C.

Production of virus free nymphs : Viviparus adults were starved for 8 h at room temperature and placed on a detached leaf of appropriate healthy host plant in a petri dish. A moistened filter paper was kept inside the petri dish to keep the atmosphere inside the petri dish humid. Newly born nymphs were transferred to fresh plants.

Aphid transmission : Aphids were collected from the plant on which they were bred with the help of a moistened brush. For fasting they were kept in a glass vial covered with a muslin cloth.

In one set of experiments aphids were allowed to starve for 2 h and then placed in groups of ten on an infected plant. They were allowed an acquisition access time of 1 min on the plant and then transferred carefully with a moistened brush to a healthy plant and allowed an inoculation acess time of 1 min. They were then killed by Andrex spray. In the other set of experiments aphids in group of ten were allowed directly, an acquisition access time of 60 min on an infected plant and an inoculation access time on a healthy plant of 12 h. Aphids were then killed by Andrex spray. All the plants were placed on a glasshouse bench and observed for a period of 6 weeks for development of symptoms.

White fly (Bemisia tabaci) : White flies were collected from N. tabacum plants growing in the field. They were multiplied on N. rustica, Glycine max, Althaea rosea, Lycopersicon lycopersicum and Zinnia elegans. Multiplication of white flies and generation transfer was done to make them virus free owing to their ability of carrying two viruses simultaneously.

Transmission studies were done using the method of Verma (1963). A period of 24 h was allowed for acquiring the virus and 48 h for inoculation.

Graft transmission : Graft transmission studies were carried out using N. tabacum cv. Anand-3. Five replicates, each having 10 plants were selected for grafting purpose. Plants of uniform growth used as stock were inoculated with the virus 15 days before grafting. Upper part of the stock was removed with a razor and a downward slit was made in the centre of the cut stem. All leaves of the stock except some lower ones were removed. The stem of the plant used as a scion was severed a few inches from the top and trimmed to a wedge shape and all leaves except the bad leaves were removed. The depth of the slit in the stock was kept equal to the length of the wedge in the scion. The wedge was inserted in slit made in the stock and was bound in place with polyethylene ribbon. The operation was carried out swiftly to avoid drying of the cut surface. Plants were

placed in a moist chamber until the graft healed.

Soil transmission : Soil transmission ~~ext~~ studies were carried out using the soil of the experimental plot where infected plants of Solanum amotapense were growing. Clay pots were filled with a mixture of clay, sand and manure in two replicates. One of the replicates was autoclaved at 20 lb pressure for 2 h and to the second, soil from the experimental plot in which infected plants were growing was added. The autoclaved replicate served as a control. Seeds of N. tabacum cvs. Anand-3, N.P. 37, GF4, Bhopali and Harrison's Special were sown in both the sets of pots. Plants raised in these pots were kept under observation for 2 months.

Dodder transmission : Seeds of Cuscuta reflexa were collected locally and grown in the glasshouse in pots of 12" diameter having autoclaved soil. Plant on attaining 6" length were trained on Nicotiana tabacum cv. Anand-3. After establishment of dodder, plants were inoculated with the virus. Fifteen days after inoculation rapidly growing end of the plant was trained on another healthy plant of the same cultivar. Dodder plant was trained by giving anticlockwise turning on the Nicotiana plant. Experiment was repeated thrice. Plants were studied upto 21 days after establishment of dodder.

Nematode transmission : Longidorus sp. was isolated from samples which was subjected to Cobbs' sieving method and the nematodes isolated by Baerman's funnel technique. The nematodes thus isolated were inoculated to the virus infected plants of N. tabacum cv. Anand-3 in proportion to 1000/kg soil. After allowing the nematodes to have an acquisition access time of 15 days on the infected plants the soil from the pots was sieved again and nematodes collected. These were then used in inoculation of healthy plants of the same cultivar and GF4 by slightly exposing the roots and pipetting the nematode over the roots and covering the roots with the same soil. Plants were observed upto 5 weeks for symptom appearance.

Properties of the virus in plant sap : Dilution end point, thermal death point and longevity in vitro was determined using essentially the technique of Noordam (1973). These studies were carried out on local lesion assay host C. amaranticolor as well as on systemic host, Nicotiana tabacum cv. Anand-3.

Sap was extracted from leaves of N. tabacum inoculated 14 days earlier using neutral 0.01M phosphate buffer in a ratio of 1g:1ml. Infected tissue was macerated in a mortar with pestle and the macerate passed through two

layers of chees cloth. The sap thus collected was used in these studies. Inoculations were done manually and inoculated leaves were rinsed with water soon after inculation.

Standardization of local lesion host for virus assay : Standardization of local lesion hosts was done by inoculating them with the standard extract. Lesions were counted 5 days after inoculation. Average number of lesion were considered before final selection of the host.

Effect of pre and post-inoculation darkening : To study the effect of pre and post-inoculation darkening . 7 sets comprising 12 plants each were used. One set was kept under normal day light conditions. The other six sets were kept either before or after inoculation in the glass house covered with cages which had all the sides and the top covered with thick black paper. Inoculation was done with the standard extract. Assay was done on C. amaranticolor.

Effect of plant size : To study the effect of plant size and number of leaves on lesion formation, plants of different size, sown at one week intervals having 3, 6, 8 and 12 leaves each were used. They were inoculated with the standard extract. Lesions were counted 5 days after inoculation.

Purification : Low speed centrifugation was performed in Sorvall RC-5B refrigerated superspeed centrifuge using SE 12 and SA 600 rotors. High speed sedimentation was performed in Sorvall OTD 65B ultracentrifuge at 40,000 rpm using A 841 rotor.

Clarification : Carbon tetrachloride, chloroform, n-butanol and a mixture of n-butanol and chloroform in equal amounts was added to the extract. In one experiment equal amount of extract and organic solvents was used and in other experiments % organic solvents were added to this extract to make concentration of 5 or 10 per cent. Addition of solvents was done slowly with continuous stirring. The extract after addition of organic solvent was allowed to stand for 30 min. During this period the mixture was shaken continuously with a magnetic stirrer. The emulsion was broken by centrifuging for 10 min at 7,000 rpm. The mixture separated in two phases with some denatured material in the interphase. The aqueous phase formed the top phase which was taken up with the help of a pipette and assayed.

Freezing and thawing : Infected leaves of N. tabacum Anand-3 inoculated 13 days earlier were harvested and kept in a polythene bag at -20°C for 24 h. The material was then allowed to thaw at room temperature and standard extract was made from this tissue. Equal amount of tissue was

harvested from N. tabacum cv. Anand-3 plants inoculated 13 days earlier and standard extract made from this tissue. Both the extracts were then assayed on C. amaranticolor.

Salt precipitation : Precipitation of Solanum amotapense virus was attempted using ammonium sulphate. Infected tissue (200g) was harvested from N. tabacum cv. Anand-3 plants inoculated 14 days earlier. The material was macerated in a mortar with pestle after addition of 0.01M phosphate buffer pH 7.0. The macerate was passed through two layers of cheese cloth. The sap thus obtained was centrifuged for 10 min at 7,000 rpm. Ammonium sulphate was added to the extract to make a concentration of 30 per cent. Ammonium sulphate was added slowly with continuous stirring. The mixture was allowed to stand for 4 h. After this period the mixture was centrifuged for 10 min at 7000 rpm. The pellet was suspended in 0.01M phosphate buffer pH 7.0 and subjected to a low speed centrifugation. The supernatant was assayed on C. amaranticolor.

Density Gradient Centrifugation : The last traces of contaminating host material were removed by rate zonal density gradient centrifugation on sucrose columns. Gradient columns were prepared by layering 40,30,20 and 10 per cent sucrose solutions prepared in neutral 0.01M phosphate buffer. These solutions were layered using a pipette with

a large orifice. Columns were kept in a referigerator and used 24 h after layering. Virus suspension (0.5ml) was loaded on top of the columns and 0.5 ml of neutral phosphate buffer was overlaid on top of it. The columns were centrifuged in a Sorvall OTD 65B ultracentrifuge at 25,000 rpm for 2 h using TST 41.14 rotor. After centrifugation the tubes were examined in a dark room by projecting a strong beam of light from the top of the tubes. The light scattering band was removed with the help of a hypodermic needle.

Analytical ultra-centrifugation : Sedimentation coefficient of the virus was determined by employing analytical ultra-centrifuge, Beckman Model E equipped with Schlieren optics and rotor type An-D SER No. 2402 with the single sector cell. Drive was supplied a constant acceleration. Virus suspension was in 0.01M phosphate buffer. The centrifuge was run at 17000 rpm. Photographic timer was set at intervals of 4 min. Boundary curve was visually observed before starting timer. Sedimentation rate (S-rate) was calculated by applying the following formula.

$$S = 1/w^2 r \; dr/dt$$

$$w = 2\pi/rpm/60$$

s = Sedimentation rate

w = angular velocity

r = position of boundary

t = time difference

Ultra violet absorption spectrum : Ultra-violet absorption studies were done by employing Pye-Unicam SP-500 uv/vis Spectrophotometer. Light scattering band removed from density gradient tube was mixed with neutral phosphate buffer of 0.01M ionic strength and subjected to high speed centrifugation (40,000 rpm for 2 h). Pellet obtained was dissolved in the same buffer and used to study UV absorption.

Electron microscopy : Dip preparation were prepared by slicing a leaf of N. tabacum cv. Anand-3 exhibiting typical symptoms with a sharp knife and dipping the cut egge in neutral 0.01M phosphate buffer. The specimen was prepared by placing a small droplet from the suspension on a formvar coated copper grid to which was added a drop of 2 per cent potassium phosphotungustate pH 6.5. After 2 min the fluid was sucked off with the help of filter paper and the grid examined in a Philips EM-400 electron microscope. Similarly, specimen was prepared from purified virus preparation obtained from density gradient centrifugation, stained negatively with potassium phosphotungustate and examined in the electron microscope.

Serology : Purified virus preparation obtained from two cycles of differential centrifugation was used for the preparation of antiserum. One year old albino rabbit was used for preparing antiserum. One ml of virus suspension

was injected in marginal vein of the rabbit. This was followed by two more intraveinous injections of 2 ml each at weekly intervals. This was followed by two intramuscular injections of virus suspension emulsified with equal amount of incomplete Freund's adjuvant. The intramuscular injection were given at intervals of 2 weeks. Samples were collected after 2 weeks of last intramuscular injection.

Serum Collection and Storage : Rabbits were fasted for 12 h prior to bleeding. A small cut was made in the marginal vein of the ear directing pinna towards ground. About 20 ml of blood was collected. Blood samples were allowed to coagulate for 2 h at room temperature and then placed in a refrigerator. Next day serum was decanted and subjected to low speed centrifugation. Straw colored liquid was collected. This antiserum was tested against purified virus and later stored in a referigerator after addition of equal amount of glycerine.

Serological tests : Tube precipitin test, chloroplast agglutination test and ouchterlony double diffusion tests were made to study antigen antibody reaction.

Tube precipitin test : Tube precipitin tests were performed in small glass tubes (Pyrex). Standard extract was subjected to low speed centrifugation. Supernatant was used as antigen.

to fold dilutions of antiserum was prepared with normal saline whereas two fold dilution of antigen was made with distilled water. One ml of each reactant was pipetted into the tube and the contents mixed and incubated at 37°C in a water bath for 2 h.

Chloroplast agglutination test : To study the chloroplast agglutination test, five drops of crude extract from infected plant was mixed with 2 drops of antiserum on a microscope slide. Clumping of chloroplasts was observed visually and with a light microscope.

Agar-gel double diffusion test : Agar-gel double diffusion tests were performed using the method of Ouchterlony (1962). One per cent agar was prepared in distilled water. Petri dishes were used for testing. Agar was flooded in the dishes so as to make a bed about 3 mm thick. Holes having a diameter of 4 mm were punched with the help of a cork borer. A central well surrounded by 4 wells were punched in the agar and the agar so cut out removed with the help of an aspirator. The distance between the walls of the wells was 4 mm. Petri dishes were incubated at room temperature and results recorded after 24 h. Different dilutions of antigen and antiserum were tested and tests were repeated several times. Proper controls were used to avoid any misreading of the

results. Double diffusion tests were also performed on slides where wells punched were of a diameter of 3 mm and the distance between the walls of the wells was also 3 mm.

Heterologous reactions : Heterologous serological tests were performed with the following antisera of some known viruses.

Antisera	Sources
Pumpkin mosaic virus	IARI, New Delhi
Tobacco mosaic virus	"
Cowpea mosaic virus	"
Bottle-gourd mosaic virus	"
Cucumber green mottle mosaic virus	"
Sunnhemp mosaic virus	"

CHAPTER 4

HOST RANGE AND SYMPTOMATOLOGY

To investigate the host range of the virus 80 plant species distributed among 17 families were inoculated mechanically using sap from plants which had been inoculated 14-15 days earlier. Plants were inoculated at least thrice to avoid misreading the results due to inoculation failure. Back inoculations were made from all inoculated plants whether exhibiting symptoms or not on Chenopodium amaranticolor - a good assay host of the virus. Symptoms on most of the plant appeared earlier in summer and were more pronounced.

Table 1 : Experimental host range of Solanum anotabense mosaic virus.

Species	Symptoms		Back inoculation
	Systemic	Local	
1	2	3	4
Amaranthaceae			
Achyranthes aspera L.	-	-	-
Amaranthus caudatus L.	-	-	-
A. viridis L.	-	-	-
Celosia cristata L.	-	-	-
Apiaceae			
Carum copticum Hiern.	-	-	-

1	2	3	4
Cuminum cyminum L.	-	-	-
Coriandrum sativum L.	-	-	-
Daucus carota L.	-	-	-
Foeniculum vulgare Mill.	-	-	-
Apocynaceae			
Catharanthus roseus (L) G. Don	-	-	-
Caryophyllaceae			
Dianthus caryophyllus L.	-	-	-
Caricaceae			
Carica papaya L.	-	-	-
Chenopodiaceae			
Beta vulgaris L.	-	-	-
Chenopodium amaranticolor Coste & Reyn	-	Necrotic local Lesion (Fig. 2)	-
C. album L.	-	Necrotic local lesion	-
C. murale	-	Necrotic local lesion	-
Kochia indica Roth.	-	-	-
Spinacea oleracea L.	-	-	-
Compositae			
Ageratum conyzoides L.	-	-	-
Calendula officinalis L.	-	-	-
Pacific beauty mixed			

1	2	3	4
Chrysanthemum coronarium L.	-	-	-
Cosmos bipinnatus Cav.	-	-	-
Dahlia variabilis L.	-	-	-
Eclipta alba L.	-	-	-
Gaillardia pulchella Fouger	-	-	-
Helianthus annuus L.	-	-	-
Sonchus asper (L) Hill	-	-	-
Tagetes erecta L.	-	-	-
Tridex procumbens L.	-	-	-
Xanthium strumarium L.	-	-	-
Zinnia elegansa Jacq.	-	-	-
Cruciferae			
Brassica oleracea L.	-	-	-
var. capitata	-	-	-
cv. Drumhead	-	-	-
cv. Pride of India	-	-	-
cv. Large early drumhead 8	-	-	-
cv. Cabbage yard long	-	-	-
var. Botrytis	-	-	-
cv. Indian snowball	-	-	-
cv. Snow ball - 16	-	-	-
cv. Super snow ball	-	-	-
cv. Purple top white globe	-	-	-
cv. Cauliflower late snow ball	-	-	-
cv. Cauliflower late maghii	-	-	-

1	2	3	4
Iberis amara L.	-	-	-
Raphanus sativus L.			
Cucurbitaceae			
Benincasa hispida Cogn.	-	-	-
Citrullus vulgaris Schard. cv. Faizabadi	-	-	-
Cucurbita pepo DC.	-	-	-
C. maxima Duch	-	-	-
Cucumis melo L. Punjab sunehri	-	-	-
Lagenaria siceraria Standl.	-	-	-
Luffa cylindrica M. Roem.	-	-	-
L. acutangula Roxb.	-	-	-
Momordica charantia L.	-	-	-
Euphorbiaceae			
Croton bonplandianum Baill	-	-	-
Euphorbia hirta L.	-	-	-
E. pulcherrima Wild	-	-	-
Phyllanthus simplex Retz.	-	-	-
Labiatae			
Salvia officinalis L.	-	-	-
Leguminosae			
Cajanus cajan (L) Druce.	-	-	-
Dolichos lablab L.	-	-	-
Glycine max (L) Merr	-	-	-

1	2	3	4
Lathyrus odoratus L.	-	-	-
L. sativus L.	-	-	-
Phaseolus vulgaris L.	-	-	-
P. radiatus L.	-	-	-
Pisum sativum L.	-	-	-
Vicia faba L.	-	-	-
Vigna sinensis (L) Savi	-	-	-
Liliaceae			
Allium cepa L.	-	-	-
A. sativum L.	-	-	-
Asphodelus tenuifolius Cav.	-	-	-
Malvaceae			
Abelmoschus exculentus Moench.	-	-	-
Althaea rosea Cav.	-	-	-
Malvastrum coromandelianum (L) Garke	-	-	-
Sida cordifolia L.	-	-	-
Polemoniaceae			
Phlox drummondii L.	-	-	-
Ranunculaceae			
Delphinium ajacis L.	-	-	-

1	2	3	4
Solanaceae			
Capsicum annuum L.			
var. Chilli G-3	Mild mosaic	-	+
var. N.P. 46-A	Mild mosaic	-	+
var. Jawala	Mild mosaic(Fig.3)	-	+
var. PC-1	Mild mosaic	-	+
C. frutescens L.	Vein clearing, mottling, stunting(Fig.4)	-	+
Datura stramonium L.	Necrotic local lesion (Fig 5)	-	-
Lycopersicon lycopersicum Mill.			
cv. tomato ruby	Mosaic	-	+
cv. tomato marglove	Mosaic (Fig. 6)	-	+
Nicotiana clevelandii Gray.	Young plants died after 3 days of inoculation old plants survived upto 15 days and then succumbed	-	-
N. glutinosa L.	Necrotic local lesion develop after 6 days of inoculation in winter At higher temp. (35°C and above) no local lesions develop but the virus becomes systemic causing mosaic and growth reduction. (Fig. 7, 8, 9).	+	-
N. plumbaginifolia Viv.	Mild mosaic developed after 13 days of inoculation in winter and after 7 days in summer and cutting of lomina (Fig.10)	-	+

1	2	3	4
N. rustica L.	Necrotic lesions developed after 7 days of inoculation in winter and after 4 days in summer (Fig.11 a+b).	+	-
N. tabacum L. cv.NP37	Dark green mosaic developed along vein 7 days after inoculation in summer and after 13 days in winter. Disappearance of chlorophyll of interveinal region (Fig.12).	-	+
N. tabacum L. cv. Anand-3	Dark mosaic developed along vein 7 days after inoculation in summer and 12 days after in winter (Fig. 13).	-	+
N. tabacum cv. GT_4	Dark green mosaic along veins developed after 7 days of inoculation in summer and 13 days in winter Disappearance of interveinal chlorophyll, distortion and stunted growth (Fig.14).	-	+
cv. Bhopali	Dark green mosaic in along virus after 7 days of inoculation in summer and 13 days in winter distortion and stunted growth (Fig.15).	-	+
cv. Harrison's special	Dark green mosaic developed after 7 days of inoculation in summer and 13 days in winter chlorophyll disappeared in the interveinal region. Vein banding, distortion and stunted growth of the plant (Fig.16,17).		
cv. White Burley	-	Necrotic local lesion (Fig.18)	-
cv. Samsun NN	Light green mosaic	-	+
cv. Xanthi	Light green mosaic	-	+

1	2	3	4
N. sylvestris Speg and Comes	-	Necrotic local lesions	-
Physalis minima L.	Mild mosaic and stunted growth	-	+
Solanum melongena L.	-	-	-
S. nigrum L.	Dark green mosaic deformation and stunted growth (Fig. 19).	-	+

- Denotes absence of symptoms; + denotes recovery of virus on back inoculation to Chenopodium amaranticolor.

CHAPTER 5

TRANSMISSION STUDIES

Aphid : Transmission studies were carried out by employing seven species of aphids viz. Aphis gossypii, A. nerii, A. fabae-solanella, A. craccivora, Acythosiphon pisum, Myzus persicae and Macrosiphonella sanbornii. Two sets each having 10 plants were used for each aphid speices. In the Ist set aphids were starved for 1.30 h followed by acquisition and inoculation access periods of one min each. In the second set acquisition and inoculation periods allowed to the aphids were 60 min and 12 h respectively. Plant were kept under similar conditions of temperature, light and humidity in the transmission chamber. Plants were kept for 6 weeks under observation. None of the plants was infected and no symptoms developed on any plant. Even increasing the number of aphid per plant from 10 to 25 did not result in the transmission of the virus. It is, therefore, concluded that the virus is not transmitted by aphids.

White flies : Transmission studies were carried out by employing white flies (Bemisia tabaci) maintained on infected Nicotiana tabacum cv. Anand-3 when white flies were transferred to healthy N. tabacum cv. Anand-3 plants no symptoms were visualized even after a lapse of one month. The same result was obtained in 5 replicates, thus indicating that the virus is

not transmitted by white flies.

Graft transmission : In all cases where the graft was successful, symptoms typical of the virus appeared on the new emerging leaves about 3 weeks after the graft union. This was found to be the only method of successful virus transmission besides mechanical transmission. Table 2 shows the results of these experiments.

Table 2 - Transmission of Solanum amotapense mosaic virus by grafting.

Replicate	Plant grafted	No. of successful grafts	No. of plants infected	Incubation period in days
1	10	5	5	21
2	10	4	4	24
3	10	5	5	21
4	10	6	6	20
5	10	3	3	21

Soil transmission : Soil transmission tests indicate that the virus is not transmitted through soil in which infected plants are growing. None of varieties of the systemically reacting species of N. tabacum viz. cvs. Anand-3, N.P. 37, GT4, Bhopali and Harrison's special exhibited any discernible symptoms.

Seed transmission : Seedlings raised from seeds obtained from infected plants remained healthy. It seems that the virus is not carried through the seeds of infected plants.

Nematode transmission : Longidorus sp. was employed to study transmission through nematodes. On inoculation N. tabacum cvs. GT_4 and Anand-3 did not show any symptoms. It was, therefore, concluded that the virus is not transmitted through Longidorus and probably is not transmitted through nematodes.

Dodder transmission : The local species of dodder (Cuscuta reflexa) was used for tranmission of the virus. Out of the 5 plants used non exhibited any symptoms. Thus indicating that the virus is not transmitted by C. reflexa. As only one species was used for transmission, no generalization can be made as to dodder transmission.

CHAPTER 6

PROPERTIES OF THE VIRUS IN PLANT-SAP

Before attempting to isolate a virus it is imperative to study its properties in plant sap. The information obtained with regard to its stability, concentration and conditions required to preserve the infectivity and maximizing the amount of virus goes a long way in its successful purification. Properties of the virus itself can be determined with great facility when preparations free from contamination of host material are obtained.

Thermal death point : Fresh sap prepared by the procedure described earlier (Chapter 3) was divided into 11 equal aliquots. Each of the aliquots was given an exposure for 10 min to a temperature in 5° steps between the range of 40 to 90°C. Inoculum was assayed on C. amaranticolor and N. tabacum cv. Anand-3. It is evident from Table 3 that the virus tolerates a temperature of 65°C but not 70°C. Thus the virus is inactivated between 65°C and 70°C.

Table 3 - Thermal inactivation of Solanum amotapense mosaic virus.

S.No.	Temperature °C	Host reaction	
		Local*	Systemic**
1.	40	120.38	+
2.	45	67.56	+
3.	50	33.23	+
4.	55	21.53	+
5.	60	18.78	+
6.	65	4.39	+
7.	70	-	-
8.	75	-	-
9.	80	-	-
10.	85	-	-
11	90	-	-

\+ = appearance of visible symptoms

\- = absence of visible symptoms

* = data based on average of 12 leaves

** = data based on 5 plants

Longevity in Vitro : Sap obtained from N. tabacum cv. Anand-3 remained infective at room temperature for 16 days and lost infectivity after a storage of 7 days. (Table 4.)

Table 4 - Longevity *in vitro* of *Solanum amotapense* mosaic virus

Temperature (^{o}C)	Storage of sap (Days)	Average number of Lesions on *C. amaranticolor**
27 ± 2	10	56.66
	11	49.29
	12	38.37
	13	24.66
	14	19.17
	15	11.23
	16	6.1
	17	-
	18	-

*Data based on average of 12 leaves

Dilution & end point (DEP) : Ten-fold dilutions of sap were prepared (10^{-1} to 10^{-7}) using 0.01 M neutral phosphate buffer. Each dilution was assayed both on *C. amaranticolor* and *N. tabacum* cv. An and-3. The virus remained infective upto a dilution of 10^{-5} but infectivity was lost at a dilution of 10^{-6}. The dilution end point of the virus lies between 10^{-5} and 10^{-6} (Table 5).

Table - 5 Dilution end point of Solanum amotapense mosaic virus

Dilution	Host reaction	
	Local*	Systemic**
10^{-1}	93.23	+
10^{-2}	68.93	+
10^{-3}	60.26	+
10^{-4}	20.52	+
10^{-5}	12.16	+
10^{-6}	-	-
10^{-7}	-	-

+ = Systemic reaction ; - = no reaction

* data based on average of 12 leaves

** data based on 5 plants

CHAPTER 7

CONCENTRATION OF VIRUS AT DIFFERENT TIMES AFTER INOCULATION

Viruses are known to attain maximum concentration in a host at a certain period after inoculation, the concentration then falls either gradually or in some cases, abruptly. It is of utmost importance to know the time after inoculation when it attains maximum concentration So that tissue from the plant may be harvested at the time when concentration is high. This varies with different viruses. To determine the time when the SaMV attains maximum concentration, three cultivers of N. tobacum viz. Anand-3, GT_4 and Bhopali which exhibit clear cut symptoms were selected. Groups of plants of each cultivar were inoculated manually and tissues harvested from randomly selected plants from each group was used for assay on C. amaranticolor. First assay was done 6 days after inoculation and then after every 24 h.

The virus attains maximum concentration in all the three cultivars 13-14 days after inoculation but among the three cultivars highest concentration, as evidenced by infectivity test, is attained in a Anand-3(Fig. 20). The virus was in N. tabacum cv. Anand-3 and for all the work tissue from the plant was harvested 13-14 days after inoculation.

CHAPTER 8

DISTRIBUTION OF VIRUS IN DIFFERENT ORGANS OF N. TABACUM cv. Anand - 3

Distribution of a virus in different organs of a plant is known to vary considerably. In such a case it is worthwhile to dissect the plant and use only those organs which contain high concentration of virus and discard others. To study the distribution of SaMV in N. tabacum cv. Anand-3 leaf, stem and root of plants inoculated 14-15 days earlier were dissected and the organs macerated in 0.01M phosphate buffer pH 7.0 followed by a low speed centrifugation. The sap thus obtained was assayed on C. amaranticolor. Table 6 shows that the roots contain no virus and a very low concentration is present in the stem whereas the bulk of the virus is in the leaf tissue.

Table 6 - Distribution of Solanum amotapense mosaic virus in different organs of N. tabacum cv. Anand-3

Part of the plant	Number of local lesion on C. amaranticolor*
Leaf	127.38
Stem	25.65
Root	None

* Data based on average of 12 leaves.

CHAPTER 9

STANDARDIZATION OF LOCAL LESION HOST FOR VIRUS ASSAY

Of all the test plants used only 5 evoked local lesions viz. *C. amaranticolor*, *C. album*, *C. murale*, *N. tabacum* cv. White Burley and *N. sylvestris*. The number of lesions on inoculated leaves of *N. sylvestris* and *C. album* were few. The lesions on *C. murale* were rather large and few in number. Both *C. amaranticolor* and *N. tabacum* White Burley were found suitable local lesion hosts but the availability of seeds and the fact that six inoculable leaves were obtained in a shorter time on *C. amaranticolor*, the latter was routinely used as an assay host in all studies. The lesions evoked were clear cut, consistent discrete and easily countable.

The optimum conditions for the appearance of local lesion on *C. amaranticolor* were determined. These include effect of pre and post-inoculation darkening, detopping, rinsing after inoculation and uniform spray or mixing of abrasive in the inoculum.

Effect of pre-and post-inoculation darkening : To study the effect of pre-inoculation and post-inoculation darkness on *N. tabacum* cv. Anand-3, seven sets of plants each having 12 plants were used. The first set served as a control, the 2nd 3rd and 4th sets were kept under darkness for 24 h, 48 h and 72 h, respectively. The fifth, sixth and seventh were kept for

24 h, 48 h and 72 h under darkness after inoculation. Lesions were counted 5 days after inoculation. On C. amaranticolor. It is evident from Table 7 that number of local lesions were enhanced when test plants were kept under darkness before inoculation for 24 h and reduced when kept 72 h darkness after inoculation.

Table 7 - The effect of pre-and post-inoculation darkening on Solanum amotapense mosaic virus.

Duration in hours	Average number of lesions*			
	Pre-inoculation darkening		Post-inoculation darkening	
	Control*	Treated*	Control*	Treated*
24	73.62	109.62	84.2	62.6
48	73.29	89.87	83.0	42.29
72	71.13	66.00	83.23	30.13

* Data based on average of 12 leaves

Effect of de-topping on lesion formation : To study the effect of de-topping two sets of N. tabacum cv. Anand-3 each having 12 plants were used. Apex of the 1st set was removed before inoculation while those of the second set remained intact. Inoculations were made on C. amaranticolor by the usual procedure. Lesions were compared after 5 days of inoculation.

Table 8 shows that detopping does not affect the number of lesions formed markedly though there is a slight increase (5.14 per cent).

Table 8 - Effect of de-topping on lesion formation by Solanum amotapense mosaic virus

Treatment	Average number of local lesions*
Apex removed	90.00
Apex intact	85.6
Control	86.30

* Data based on an average of 12 plants

Effect of carborundum : The effect of carborundum 500 (Silicon carbide) when used in different ways, on the number of local lesions was studied. Inoculum was prepared by the usual procedure and divided into 3 parts. The Ist part was inoculated directly without carborundum, 2nd part was mixed with carborundum and inoculated and the 3rd part was inoculated on leaves pre-dusted with carborundum. Inoculations were made on C. amaranticolor and it was noted (Table 9) that the method in which carborundum was dusted on leaves before inoculation gave the highest number of lesions.

Table 9 - Effect of carborundum on lesion formation of Solanum amotapense mosaic virus.

Treatment	No. of local lesion*
Control	71
Without carborundum	41
Carborundum mixed with the inoculum	56
Carborundum dusted over leaves before inoculumation	70

* Data based on average of 12 plants

Effect of plant size on lesion formation : To select correct size and number of leaves on assay host 6 plant having 3, 6, 8 and 12 fully expanded leaves on the central stem were selected for inoculation. They were inoculated by the usual method and lesions evoked were counted 5 days after inoculation. Table 10 shows that C. amaranticolor having 6 expanded leaves of equal size should be used for assay.

Table 10 - Effect of plant size on lesion formation by Solanum amotapense mosaic virus.

Leaf stage	Number of local lesions	Control
3 leaves	31.29	57.38
6 leaves	57.33	57.38
8 leaves	41.36	57.38
12 leaves	39.54	57.38

Data based on average of 12 plants

Effect of rinsing after different intervals of inoculation : To study the effect of rinsing after different intervals of inoculation, 4 sets of C. amaranticolor plants, each having 12 plants were used. Plants of the Ist set were rinsed immediately after inoculation while of the 2nd, 3rd and 4th set were rinsed 1 h, 2 h and 3 h after inoculation. Table 11 shows that lesion develop most in plants which are rinsed immediately after inoculation and when rinsing is delayed the development of lesions is reduced to some extent.

Table 11 - Effect of rinsing on lesion formation after inoculation of Solanum amotapense mosaic virus.

Treatment	Number of local lesion*
Control	110.29
Immediate rising	110.72
1 h after inoculation	104.5
2 h after inoculation	95.5
3 h after inoculation	89.25

* Data based on average of 12 plants

CHAPTER 10

PRELIMINARY STUDIES ON PURIFICATION

Effect of buffers : Young infected leaves of *N. tabacum* Anand-3 were macerated in various buffers separately. Inoculum was prepared by usual method of preparation and assayed on *C. amaranticolor*.

Table 12 - Effect of various buffers at different pH values on the infectivity of *Solanum amotapense* mosaic virus

Buffer	Molarity	pH	Number of local lesion/leaf on *C. amaranticolor*	Control
Citrate	0.1M	6.00	27.25	170.39
		6.50	42.25	
		7.00	40.75	
Borate	0.2M	6.7	104.5	
		6.09	90.0	
		7.36	75.0	
Borate - NaOH	0.1M	9.3	47.75	170.39
		9.44	69.25	
		9.67	52.87	
Phosphate - Borate	0.1M	8.2	94.25	190.39
		8.4	103.62	
		8.6	118.5	
Glycine HCl	0.01M	4.5	29.25	170.39
		5.0	41.25	
		5.5	52.25	
Carbonate bicarbonate	0.1M	6.00	26.25	170.39
		6.5	40.25	
		7.0	36.03	
Phosphate	0.01M	6.0	108.92	170.39
		6.5	134.61	
		7.00	166.88	

* Data based on average of 12 leaves

Of all the seven buffers used at different pH levels 0.01M phosphate buffer pH 7.0 gave the highests number of local lesions. The number of local lesion in this case were about the same as in the control (Table 12). So in all studies 0.01M phosphate buffer pH 7.0 was routinely used.

Ionic Strength : To determine the most suitable ionic strength of phosphate buffer for retaining the infectivity of the virus 0.01M, 0.1M and 0.2M buffers were used for macerating the infected tissue and the sap thus obtained was assayed on C. amaranticolor. It is evident from Table 13 that phosphate buffer pH 7.0 having 0.01M ionic strength is most suitable for retaining infectivity of the virus.

Table 13 - Effect of phosphate buffer on the infectivity of Solanum amotapense mosaic virus

Buffer	Molarity	pH value	Average number of local lesion on C. amaranticolor*	Control
Phosphate	0.01M	6.00	108.92	170
		6.5	134.61	
		7.0	161.88	
	0.1M	6.00	74.55	"
		6.50	93.10	
		7.00	100.8	
	0.2M	6.00	35.00	"
		6.50	37.25	
		7.00	47.75	

* Data based on average of 12 leaves.

Additives : For stabilizing and enhancing stability of the inoculum, some chelating compound e.g. diethyl dithiocarbomate and anti-oxidiging agent, sodium sulphite were added to the inoculum.

Effect of diethyl dithiocarbamate and sodium sulphite : To study the effect of diethyl dithiocarbamate (DIECA) and sodium sulphite, sap prepared from infected plant was divided into 3 aliquots. The first aliquot was kept as control (untreated), the 2nd and 3rd aliquots were mixed with 0.01 per cent sodium sulphite and .01 M diethyl dithiocarbamate. These were allowed to stand for 30 min. Samples were assayed on C. amaranticolor. Lesions were counted 5 day after inoculation. It is evident from Table 14 that both sodium sulphite and diethyl dithiocarbamate increase the infectivity of the virus. The increase in lesion number being more significant with diethyl dithiocarbamate.

Table 14 - Effect of diethyl dithiocarbamate and Sodium chloride on the infectivity of Solanum amotapense mosaic virus.

Treatment	Number of local lesion on C. amaranticolor*
Control	160.13
Sodium sulphite	190.85
Diethyl dithiocarbamate	213.66

* Data based on average of 12 leaves

Effect of sodium chloride, sodium sulphite and diethyl-dithiocarbamate : To study the combined effect of sodium chloride, sodium sulphite and diethyl dithiocarbamate, sap from infected plant was divided into 3 aliquots. The first aliquot was used as control, the 2nd aliquot was mixed with 1 per cent sodium chloride, 0.01 per cent sodium sulphite and 0.01 M diethyl dithiocarbamate and the 3nd aliquot was mixed with similar quantities of aforesaid chemicals and subjected to low speed centrifugation. The chemicals were mixed and stirred for 30 min. Each aliquot was assayed on C. amaranticolor and lesions were counted 5 days after inoculation. Table 15 indicates that when soium chloride, sodium sulphite and diethyl dithiocarbamate was used in combination there was an increase in number of lesions. This increase is about the same when only diethyl dithiocarbamate is used. Low speed centrifugation after the treatment results in some loss of the virus.

Table 15 - Effect of sodium sulphite, sodium chloride and diethyl dithiocarbamate on the infectivity of the Solanum anetapense mosaic virus

Treatment	Number of local lesion on C. amaranticolor*
Control	160.66
Sodium chloride, + Sodium sulphite + Diethyl dithiocarbamate	219.39
Sodium chloride + Sodium sulphite + Diethyl dithiocarbamate followed by low-speed centrifugation	207.38

* Data based on average of 12 leaves.

CHAPTER II

PURIFICATION

To study the properties of a virus specially its biophysical, bio-chemical and immunological properties, it is essential to obtain preparations which are free from host material and still retain infectivity. Identification of particles is possible in preparations which are free from contaminants. It is imperative to establish correlationship between the identified particles and infectivity. Obtaining preparations which are free from host contaminants and normal plant proteins, without loss and inactivation of virus is often a difficult problem. Before attempts are made to isolate the virus itself from infected tissue, it has to be extracted from infected tissue and the extract so obtained clarified.

Extraction : Leaves from infected plants showing characteristic symptoms inoculated 15 days earlier were harvested and the tissue disrupted in a blender using 0.01M phosphate buffer pH 7.0 containing 0.1M diethyl dithiocarbamate, 1 per cent sodium chloride and 0.01 per cent sodium sulphite. The slurry was passed through double layer of cheese cloth and the sap obtained was centrifuged for 10 min at 7000 rpm. The pellet was discarded and the supernatant used as starting material for purification.

Clarification

Organic Solvents: Clarification of the crude extract to remove most of the contaminating host material was attempted by addition of some organic solvents, triton x-100 and by freezing and thawing infected tissue.

Effect of carbon tetrachloride, chloroform, n-butanol and a mixture of n-butanol and chloroform in equal volumes was investigated in one experiment. Preliminary experiments revealed high losses in infectivity when these organic solvents were used in equal volumes with the extract so 5 and 10 per cent of these organic solvents were used. The solvents were added slowly to the extract with continuous stirring and the suspension allowed to stand for 30 min. The emulsion was broken by a low speed centrifugation (10 min at 7000 rpm). Table 16 shows that 10 per cent carbon tetra chloride, chloroform and n-butanol affected the infectivity adversely. A mixture of n-butanol and chloroform maintained the infectivity of the virus to a great extent and normal constituents of the host and pigments were removed to a greater extent as compared to other solvents.

Table 16 - Effect of organic solvents on the infectivity of *Solanum anomalense* mosaic virus.

Solvent	Percentage	local lesions on *C. amaranticolor*
Control	-	176.3
Carbon tetrachloride	5	157.3
	10	67
Chloroform	5	139.2
	10	53.1
n-butanol	5	68.8
	10	45
Mixture of n-butanol + chloroform	10	170.8

* Data based on average of 12 leaves

4. Triton X-100

Though used primarily in the initial extraction medium to assist in the release of virus particles from cell component, it may also assist in clarification of the crude extract.

The crude extract was divided into two aliquots. The first was allowed to stand for 30 min. To the second Triton

X-100 was added to bring it to a concentration of 5 per cent. This was allowed to stand for 30 min with constant stirring. After this period both were given a low speed centrifugation (10 min at 7000 rpm. Supernatant obtained was assayed on C. amaranticolor.

In three different experiments as evidence by Table 17 infectivity was found to be enhanced by Triton X-100. The increase in infectivity in all the three experiments was more than 20 per cent.

Table 17 - Effect of Triton X-100 on the infectivity of Solanum amotapense mosaic virus

Treatment	Percentage	Number of Lesion on C. amaranti-color	Control	Percentage increase
Experiment 1	5	67.53	56.23	
" 2	5	74.31	62.56	21.45
" 3	5	74.43	59.29	

* Data based on average of 12 leaves

Freezing & thawing : Freezing infected tissue facilitates removal of host material for viruses which are stable. In

certain cases it has proved to effect infectivity adversely when infected tissue was frozen for 24 h before extraction of Solanum amotapense mosaic virus, infectivity was reduced to a great extent as is evident from table 18.

Table 18 - Effect of freezing and thawing on the infectivity of Solanum amotapense mosaic virus.

Treatment	Number of local lesion on C. amaranticolor*	Percentage
Freezing & thawing	118	25
Untreated	157	

* Data based on average of 10 leaves

Low speed centrifugation : Crude extract was centrifuged at low speed at different rpm to see its effect on infectivity as well as clarity of the extract. The uncentrifuged extract served as control. Data included in table 19 shows that highest infectivity is present in extracts centrifuged at 5,000 rpm. Centrifugation at 7000 rpm reduces infectivity to a slight extent but considerable amount of plant material is got rid off. After centrifugation at 10,000 rpm infectivity is impaired to some extent. In all future work for clarification preparations were centrifuged at 7000 rpm for 10 min.

Table 19 - Effect of low speed centrifugation on the infectivity of the *Solanum amotapense* mosaic virus

Revolutions per minute	Duration	Number of average local Lesions on *C. amaranticolor*	Control
5000	10 min	133.3	136.66
7000	10 min	124.20	136.66
10000	10 min	119.23	136.66

* Data based on average of 12 leaves

CHAPTER 12

ISOLATION OF VIRUS FROM CLARIFIED EXTRACT

It is unlikely that a single isobation procedure would work with equal effectiveness for all viruses due to the properties of the virus, nature of host and several other factors. Generally employed purification procedures fall into 3 groups : 1. adsorption 2. Precipitation 3. Differential centrifugation.

Adsorption techniques are generally used in conjuction with other methods in purification and concentration of viruses. This technique could not be used due to lack of facilities.

Salt precipitation : Precipitation by ammonium sulphate has been used to purify tobacco mosaic, tomato bushy stunt, tobacco necrosis, turnip yellow mosaic and several other viruses. Application of this technique failed to precipitate the virus in these studies. Preparations obtained showed no infectivity.

Fractionalism with polyethylene glycol : Precipitation of viruses with polyethylene glycol (PEG) was used for a number of viruses (Hebert, 1963; Lebermann, 1966; Gooding and Hebert, 1967; Albrechtova and klir, 1968; Mahmood and Peters 1973). To sap extracted from infected tissue was added sodium chloride to make a concentration of 0.2M. This extract containing sodium

chloride was divided into 4 aliquots to which PEG 6000 was added to make concentration of 2, 4, 6 and 8 per cent, respectively. These were allowed to stand for 30 min with continuous stirring followed by a low speed centrifugation. Pellet was suspended in .01M phosphate buffer pH 7.0 and centrifuged for 10 min at 7000 rpm. Supernatant obtained in each case was assayed on C. amaranticolor. No lesions were observed on any test plant. It seems that Solanum amotapense mosaic virus is not precipitated under the conditions employed.

Differential centrifugation : Differential centrifugation has s to a great extent supplanted the earlier techniques. It involves the application of alternate cycles of high and low speed centrifugation. The size and density of the known viruses are such that they are pelleted from solutions within 2 h in high centrifugal fields, the aggregated contaminants being removed by low speed centrifugation. This procedure is widely used to concentrate the virus and to separate the virus from contaminants. These procedures are usually preceeded by various methods of clarification and are some times followed by density gradient centrifugation, enzymatic treatments, electrophoresis, and serological methods as adjunct to obtain highly purified preparations. Differential centrifugation was finally employed for purification of Solanum amotapense mosaic virus.

The extract obtained from infected tissue was clarified by addition of a mixture of n-butanol and chloroform (in equal volumes) to the extract to make a concentration of 10 per cent. The mixture was added slowly with vigorous stirring. It was allowed to stand for 30 min with continuous stirring. The emulsion was broken by centrifuging for 10 min at 7000 rpm. Aqueous phase was collected and centrifuged at 40,000 rpm for 2 h. Supernatant was discarded and the pellet suspended in 0.01M phosphate buffer pH 7.0 and given a low speed centrifugation. The pellet obtained was discarded and supernatant centrifuged for 2 h at 40,000 rpm. The pellet obtained was resuspended in 0.01M phosphate buffer pH 7.0 and centrifuged at 7000 rpm for 10 min. The supernatant obtained (Fig. 21) was clear opalescent and infectious and was used to study the properties of the virus.

Infectivity of the virus at different stages of the purification procedure was tested to assess loss of virus at different stages. Suspensions obtained after the 1st and IInd cycles of differential centrifugation were tested after diluting it to the extent to bring it to the original volume. Table 20 shows that high speed centrifugation affects the infectivity to some extent. Most of the loss occurs during the Ist cycle of differential centrifugation. The final virus suspension obtained still retained high infectivity.

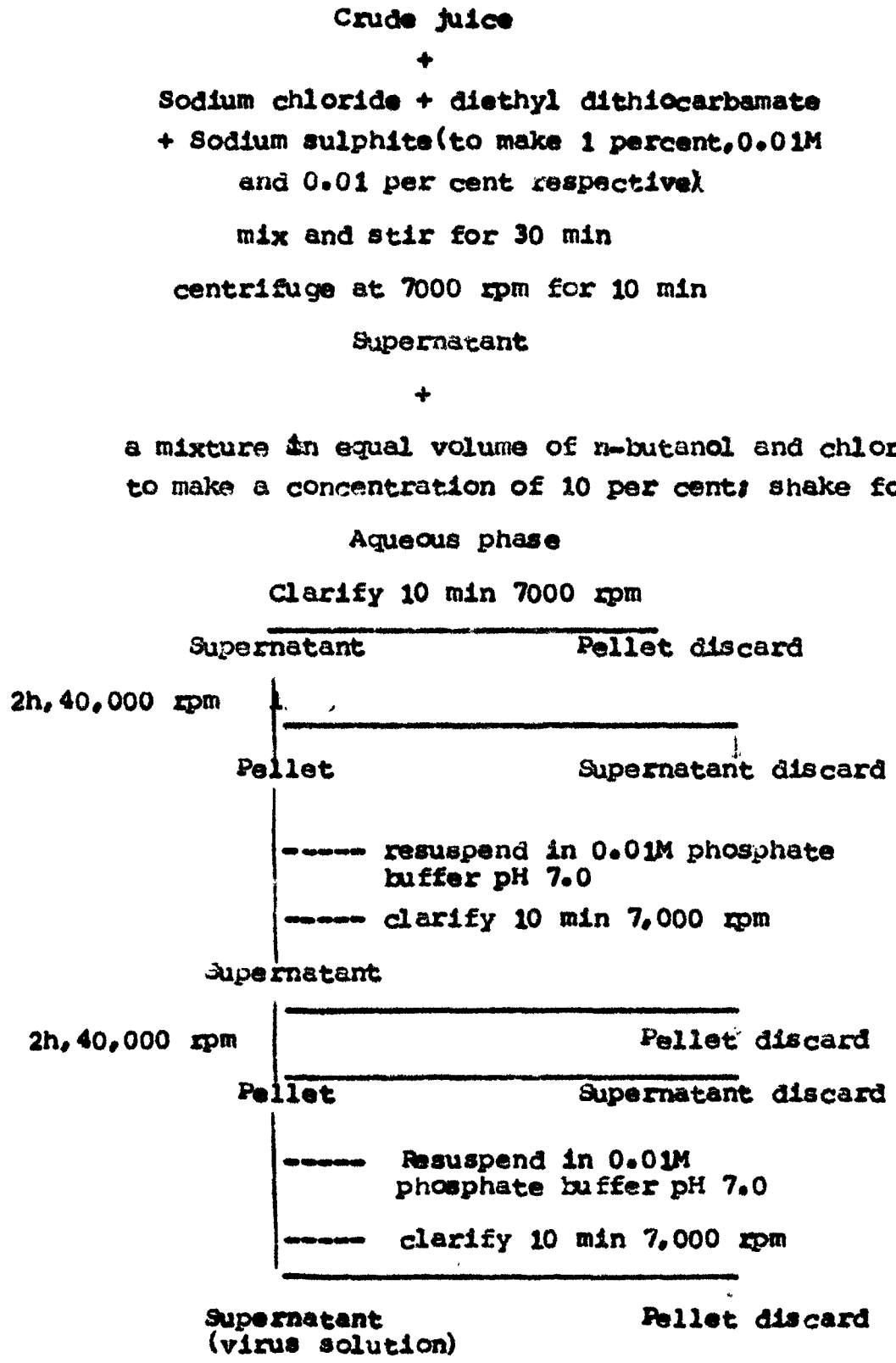

Fig. 21. Flow diagram of Solanum amotapense mosaic virus purification.

Table 20 - Infectivity of Solanum amotapense mosaic virus at different stages of purification.

Stages	No. of local lesions/leaf*
Extract	131
After Ist cycle of differential centrifugation	100
After 2nd cycle of differential centrifugation (purified virus preparation)	90

Density Gradient centrifugation : Further purification of the virus preparation was achieved by density gradient centrifugation. The last traces of plant proteins, ribosomes and other contaminants were removed by rate zonal density gradient centrifugation on sucrose columns. Preparations obtained after two cycles of differential centrifugation were loaded on sucrose columns and centrifuged for 25000 rpm for 30,60,90 min. The tubes were viewed in a dark room by projecting a narrow beam of light down the tubes from the top.

One light scattering band was visualized. The distance transvereed by the band after 30 min was 16 mm from the miniscus and after 90 min 32 mm (Fig.22). The light scattering band

was consistently present. Material removed from the light scattering band after dilution was inoculated on to the leaves of C. amaranticolor. Innumerable lesions were evoked on these leaves indicating that it was infectious.

To establish thatt the light scattering band was infact due to the virus and not an artifact or due to host components, equal amounts of healthy and infected tissue form plants of same age and size and raised under similar conditions was subjected to the pruification procedure adopted and the pellets obtained after the second cycle of high speed centrifugation after suspension in 0.01M phosphate buffer pH 7.0 was clarified by low speed centrifugation and layered on top of density gradient tubes. The tubes were centrifuged for 90 min at 25,000 rpm. in a swinging bucket rotor. A light scattering band was visualized in the tube on which material from infected tissue was layered and no such corresponding band was present in the tube on which material from healthy plants was layered. The position of the band corresponded tc those seen earlier.

CHAPTER 13

PROPERTIES OF PURIFIED VIRUS PREPARATION

Ultraviolet absorption spectrum : Purified virus preparation obtained from density gradient centrifugation, mixed in neutral 0.01M phosphate buffer was subjected to a cycle of high speed centrifugation at 40,000 rpm. for 2 h. Pellet obtained was dissolved in 0.01M phosphate buffer pH 7.0 and examined in a Pye-unicem SP8-500 UV/VIS spectrophotometer. Preparation gave a spectrum typical of nucleo-proteins(Fig. 23). Results are summarized below :

Maximum absorbance	= 258 nm
Minimum absorbance	= 246 nm
A280/260	= 0.78
A 260/280	= 1.14
A max/A min	= 1.05
A 260	= 0.54
A 280	= 0.47

The results are indicative of a low amount of nucleic acid, which was calculated to be 5 per cent.

Analytical ultracentrifugation : Sedimentation studies of the purified virus preparation were carried out with an analytical ultracentrifuge Beckman spinco Models E equipped with schlieren

optics.Boundary position was measured by Nickon projection profile device. Sedimentation coefficient was calculated to be 173S. The virus sedimented as a single peak suggesting that the preparation was homogenous and contained a single species of particles(Fig.24). It also indicated that no aggregation of particles during purification has taken place. The sedimentation results are in aggreement with that obtained by density gradient centrifugation showin one light scattering band.

Electron microscopy : Dip preparations made from infected N. tubacum cv. Anand-3 leaves revealed the presence of rigid rod shaped particles measuring 318 nm in length and 18 nm in diameter (Fig. 25).

Purified preparation obtained after density gradient centrifugation were pelleted by high speed centrifugation. The pellet was suspended in 0.01M phosphate buffer pH 7.0 and after giving a low speed centrifugation examined in the Electron microscope. These preparations also revealed the presence of rigid rods which measured 318 nm in length and 18 nm in width (Fig.26). Most of the particles were of this length though occasionally particles of varying lengths were also observed.

CHAPTER 14

SEROLOGY

Antiserum prepared against SaMV in rabbits reacted specifically with sap from SaMV-infected plants in tube precipitin tests. No precipitate was observed when normal serum or sap from healthy plants was used in these tests. A precipitate was observed upto a dilution of 1:128 of the antiserum thereby indicating that the titre of the antiserum was 128.

Purified virus and sap from infected *N. tabacum* cv. Anand-3 reacted specifically with the antiserum in agar-gel double diffusion tests. A line of precipitate was formed mid way between the antigen and antiserum wells. No lines were formed with healthy plant sap, normal serum or saline (Fig.27).

When freshly expressed sap from infected plant was mixed with the antiserum clumping of particles was observed. No such clumping occurred when freshly expressed crude sap from healthy plants was used.

SaMV was tested against antisera of SaMV, cucumber green mottle mosaic, pumpkin mosaic and tobacco mosaic virus(Johnson's strain) in agar-gel double diffusion tests. The antigen was placed in the central well and antisera in the surrounding wells. Lines of precipitate formed between the well containing SaMV and antisera against SaMV, cucumber green mottle mosaic

virus and pumplin mosaic virus and no line was formed between the well containing SaMV and antiserum against TMV-Johnson's strain (Fig.28). In another experiment antiserum against cowpea mosaic virus (TMV strain) was set up in the central well and sap from SaMV infected plant and sap from healthy plant in the surrounding wells. A line of precipitate formed only between the a antiserum well and the well containing sap from SaMV infected plant (Fig. 29). SaMV was reacted in tube precipitation test with antisera against pumpkin mosaic, tobacco mosaic, cowpea mosaic (TMV strain), bottle gourd mosaic, cucumber green mottle mosaic and sunn hemp mosaic viruses. Precipitation was observed only with pumpkin mosaic, bottle gourd mosaic and cucumber green mottle mosaic viruses. The precipitate formed with antiserum of pumpkin mosaic virus was heavier than those formed with the antisera of bottle gourd mosaic or cucumber green mottle mosaic viruses (Table 21).

Table 21 - Heterologous relationship of SaMV with antisera of some known viruses.

Antisera	SaMV Antigen	Healthy sap
Normal Serum	-	-
Pumpkin mosaic virus	+++	-
Tobacco mosaic virus (Jonhnson's strain)	-	-
Cowpea mosaic virus (TMV strain)	-	-
Bottle guard mosaic virus	++	-
Cucumber green mottle mosaic virus	++	-
Sunn hemp mosaic virus	-	-

+++ = heavy reaction ++ = Moderate reaction

\- = No reaction

CHAPTER 15

DISCUSSION

Solanum amotapense a wild plant of family Solanaceae was reported from Peru in 1946 by Svenson (1946). So far no virus disease has been reported on this plant. The present report is the first, on the natural occurrence of a virus disease on S. amotapense. Solanum amotapense mosaic virus (SaMV) which was isolated from S. amotapense caused a green mosaic on newly emerging leaves, whereas older leaves exhibited mild mosaic symptoms.

The virus is easily sap transmissible and is also transmitted by grafting. Several species of aphids viz. Myzus persicae, Aphis gossypii, A. nerii, A. craccivora, White fly (Bemisia tabaci) and nematodes failed to transmit the virus. The virus is not transmitted through the seed of infected plants.

SaMV has a limited host-range, causing systemic infection in members of family Solanaceae only. It caused dark green mosaic in N. tabacum cvs. GT4, Bhopali, Harrison's special, N.P. 37, Anand-3 and mild mosaic in Samsun NN and Xanthi. N. glutinosa reacts differently in different seasons. In winter necrotic local lesions are produced on the inoculated leaves whereas systemic infection occurs in summer when temperature is above 38°C. N. rustica, N. tabacum cv. White Burley and

N. sylvestris react with necrotic lesions. Severe hypersensitive reaction occurs on N. clevelandii. Young plants die within a week of inoculation while older ones take about 15 days. Capsicum annuum develops mild mosaic whereas C. frutescens develops vein clearing followed by mottling and stunting.

Lycopersicon lycopersicum reacts with yellow mosaic mottling. S. melongena did not react while S. nigrum developed dark green mosaic and showed stunted growth.

Besides members of Solanaceae SaMV infected three species of Chenopodiaceae viz., Chenopodium amaranticolor, C. murale and C. album causing necrotic lesion in them but no systemic invasion followed.

In plant sap SaMV remains infectious upto 65°C but not 70°C. It withstands a dilution of 10^{-6} but not 10^{-7} and when stored at room temperature retains infectivity for 16 days. SaMV attained maximum concentration in N. tabacum cv. Anand-3 13 days after inoculation. The concentration of the virus decreased from the 15th day but the decrease was gradual and even after 20 days plants contained high amount of virus as indicated by infectivity tests.

The most suitable medium to maintain the infectivity of SaMV was found to be 0.01M phosphate buffer pH 7.0. It was purified by a method involving extraction in 0.01M phosphate buffer pH 7.00 having 0.01 per cent sodium sulphite, 1 per cent sodium chloride, and 0.01M diethyl dithiocarbamate. The

mixture was clarified by adding butanol and chloroform (in equal volumes) and subjecting it to two cycles of differential centrifugation. The last traces of normal plant constituents were removed by rate zonal density gradient centrifugation. SaMV showed one light scattering band in gradient tubes. Material removed from this band was highly infectious. Purified preparation gave maximum absorbance at 258 nm and minimum at 246 nm. In analytical centrifuge it gave a single peak with sedimentation coefficient of 173S.

Purified preparations when examined in the electron microscope revealed the presence of elongated rod shaped particles 318 nm long and 18 nm wide. Such particles were also found in dip preparations from leaves of infected plants.

SaMV reacted with the antisera of pumpkin mosaic, cowpea mosaic (a TMV strain), bottle gourd mosaic and cucumber green mottle mosaic viruses and showed no reaction with antisera of TMV (Johnson's strain) and sunn-hemp mosaic virus.

SaMV has a restricted host range and reaction on most Nicotiana spp. agrees with those evoked by tomato mosaic virus. On N. sylvestris necrotic lesions are caused and no systemic invasion follows. Necrotic lesions are evoked on N. tabacum White Burley by SaMV and most Tomato mosaic virus isolates induce local lesions on this host but some also invade systemically. TCMV induced necrotic local lesions on N. glutinosa

others caused systemic mottle. SaMV induces only necrotic lesions on this host. Reaction of TOMV and SaMV is identical on N. clevelandii which is killed by the two viruses.

Reaction on D. stramonium is also identical by both the viruses. Necrotic lesions are produced on Phaseolus vulgaris by SaMV and some strains of TOMV are also reported to cause reddish lesion on this host but others do not infect. Local lesions are evoked on Chenopodium spp. by both the viruses but TOMV becomes systemic later on.

SaMV could not be transmitted by dodder. As only one species was used its transmission by this method can not be ruled out.

SaMV does not seem to be as highly immunogenic as TOMV as the titre obtained was rather low. It shows no serological relationship with TMV type strain but shows serological cross reactivity with cowpea mosaic (a TMV strain) cucumber green mottle mosaic and bottle gourd mosaic and pumpkin mosaic viruses.

Production of local lession but no systemic invasion in Nicotiana sylvestris is considered to be of diagnostic importance for TOMV strains (Wang and Knight, 1967). Reaction of SaMV on most of the solanaceous hosts suggest that it is a

strain of tomato mosaic virus.

It seems that SaMV is a hitherto undescribed strain of TOMV due to differences in the reported size of particles of other strains of TOMV and also in the sedimentation coefficient. The loss of infectivity in plant tissue when stored at low temperature and inability of SaMV to invade Chenopodium spp. Systemically is also indicative of this.

On the basis of symptomatology, host reaction, properties in crude sap, size of particle and serological behaviour SaMV is undoubtely a member of Tobamo virus group.

Fig. 1. *Solanum amotapense*, young (left) and old (right) leaves showing mosaic symptoms.

Fig. 2. *Chenopodium amaranticolor* right leaf showing necrotic lesions; left, a healthy leaf.

FIG. 1

FIG. 2

Fig. 3. *Capsicum annuum* leaves showing mild mosaic.

Fig. 4. *Capsicum frutescens*. leaves leaves showing vein clearing.

FIG. 3

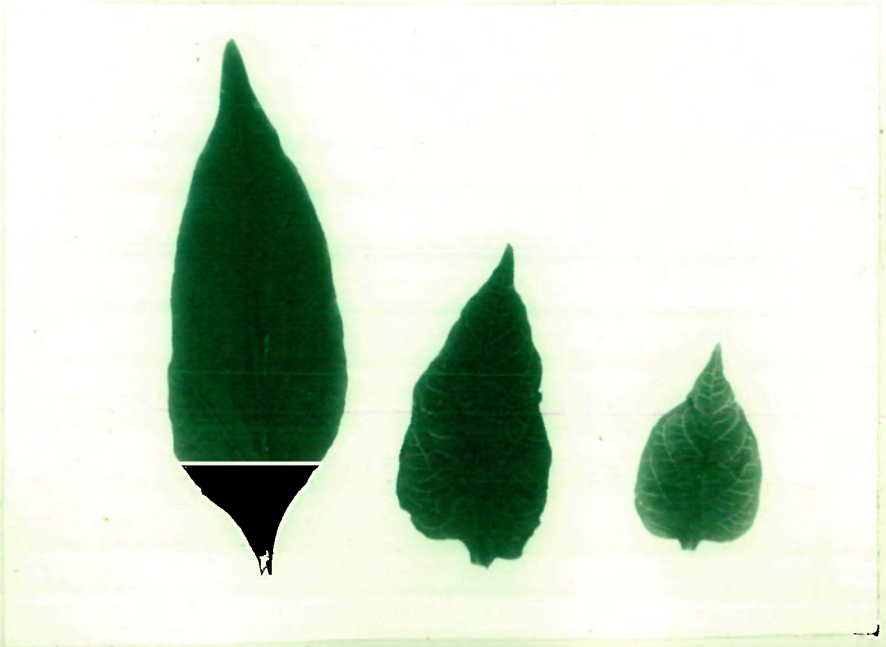

FIG. 4

Fig. 5. *Datura stramonium*. right leaf showing necrotic local lesions; left, a healthy leaf.

Fig. 6. *Lycopersicon lylopersicum* cv. marglobe right leaf showing mosaic; left, a healthy leaf.

FIG. 5

FIG. 6

Fig. 7. *Nicotiana glutinosa* right and central leaf showing necrotic lesion; left, a healthy leaf.

Fig. 8. *Nicotiana glutinosa*, right and central leaf showing mosaic, left, a healthy leaf.

FIG. 7

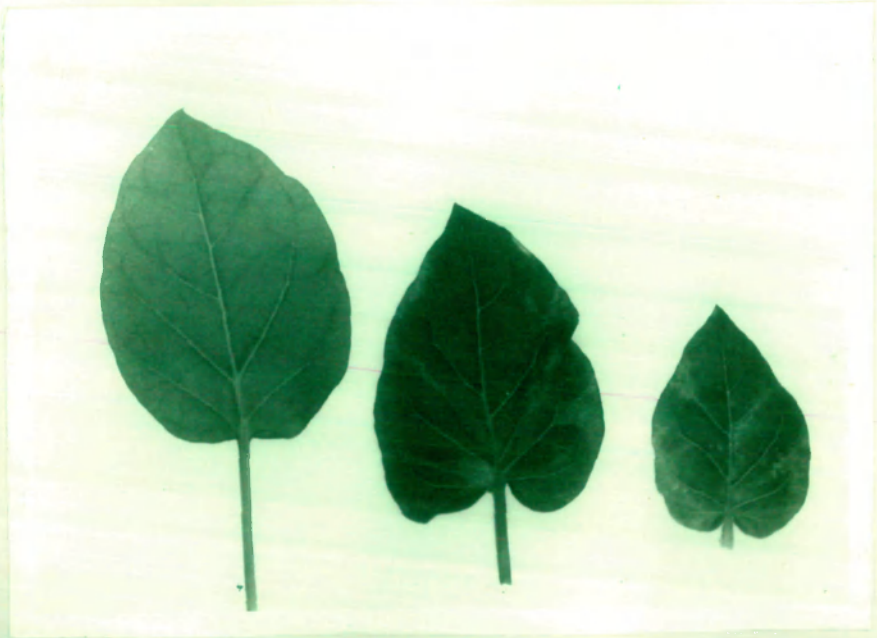

FIG. 8

Fig. 9. *Nicotiana glutinosa*, left plant showing reduction in size; right, a healthy plant.

Fig. 10. *Nicotiana plumbaginifolia*, right leaf showing mild mosaic and lamina reduction; left, a healthy leaf.

FIG. 9

FIG. 10

Fig. 11 A. *Nicotiana rustica*, a healthy leaf.

Fig. 11 B. *Nicotiana rustica*, leaf showing necrotic local lesions.

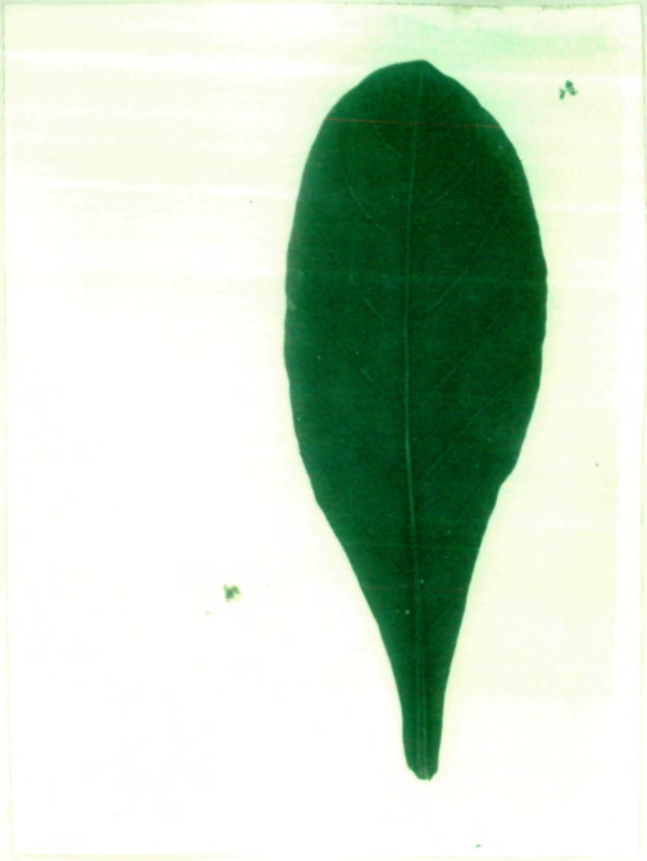

FIG. II A

FIG. II B

Fig. 12. Nicotiana tabacum cv. N.P. 37 right leaf showing mosaic; left a healthy leaf.

Fig. 13. Nicotiana tabacum cv. Anand-3, right leaf showing mosaic; left, a healthy leaf.

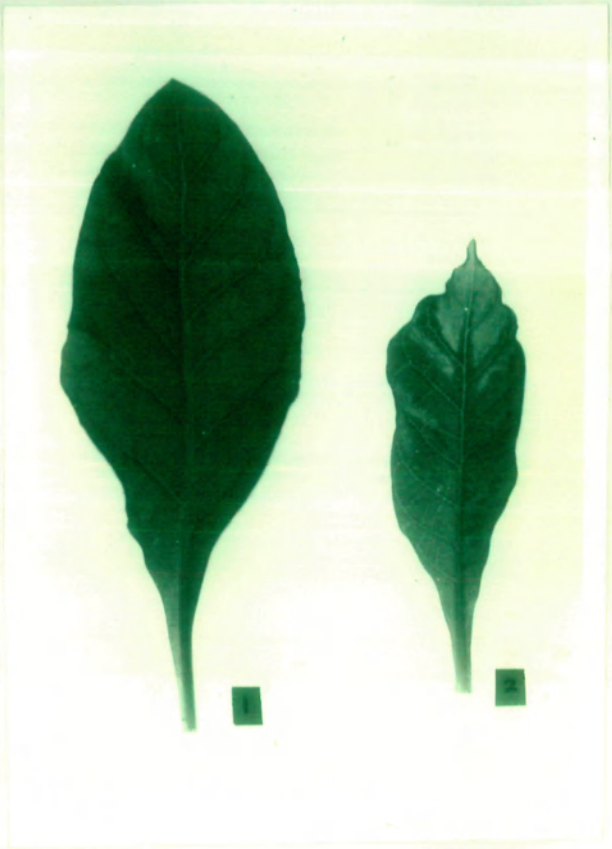

FIG.12

FIG.13

Fig. 14. *Nicotiana tabacum* cv. GT4, right leaf showing mosaic; left, a healthy leaf.

Fig. 15. *Nicotiana tabacum* cv. Bhopali, right leaf showing mosaic; left, a healthy leaf.

FIG 14

FIG 15

Fig. 16. Nicotiana tabacum cv. Harrison's special, right leaf showing mosaic; left, a healthy leaf.

Fig. 17. Nicotiana tabacum cv. Harrison's special, left plant showing reduction in size; right, a healthy plant.

FIG.
16

FIG. 17

Fig. 18. *Nicotiana tabacum* cv. white Burley, right leaf showing necrotic lesions; left, a healthy plant.

Fig. 19. *Solanum nigrum* right leaf showing deformation left, a healthy leaf.

FIG. 18

FIG. 19

Fig. 20 . Concentration of virus in different cultivars of Nicotiana tabacum cvs. Bhopali, GT4 and Anand-3 (1).

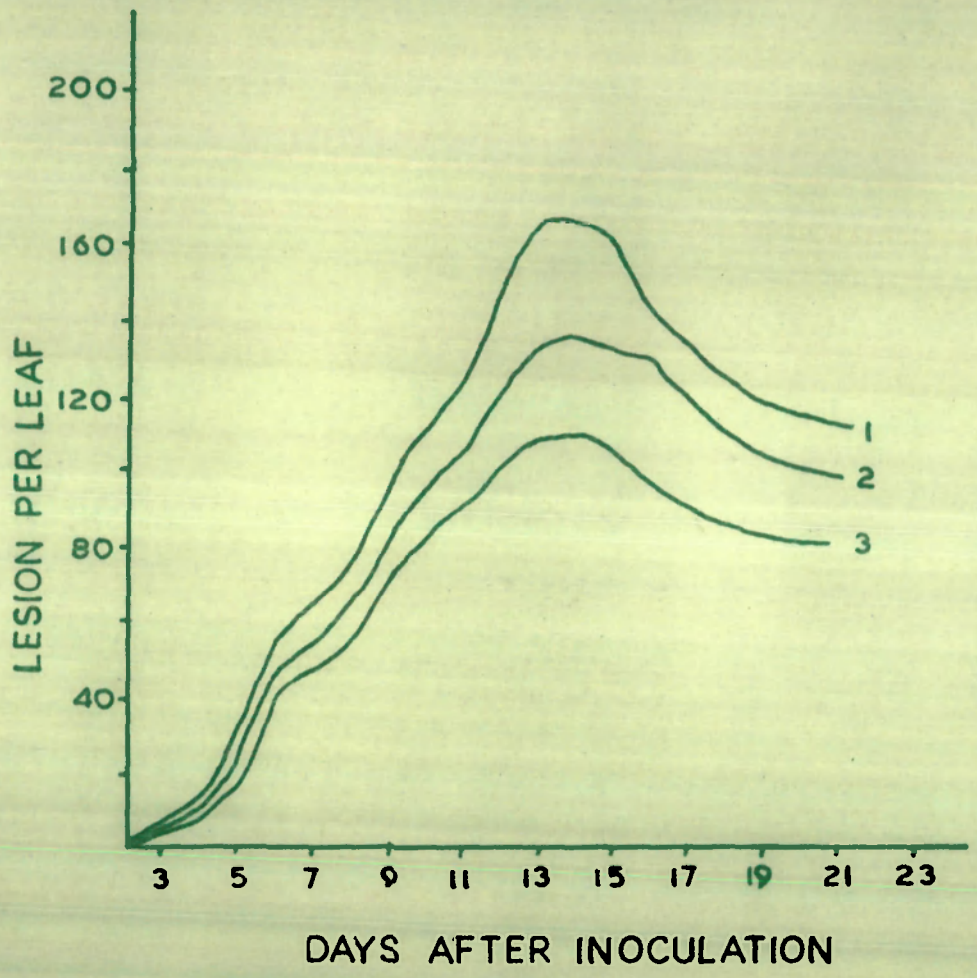
200
160
120
80
40
LESION PER LEAF
1
2
3
3
5
7
9
11
13
15
17
19
21
23
DAYS AFTER INOCULATION

Fig.20

Fig. 21. Flow diagram of purification of *Solanum* [illegible] mosaic virus(Page 94).

Fig. 22. Rate zonal density gradient centrifugation in sucrose gradient zones for 90 min at 25000 rpm.

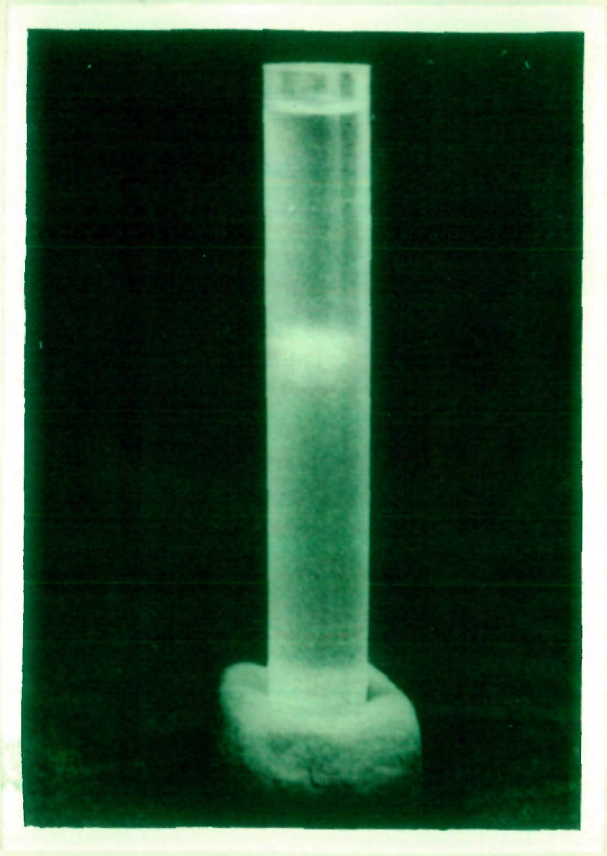

FIG. 22

Fig. 23. Ultra violet spectrum of purified *Solanum amotapense* mosaic virus.

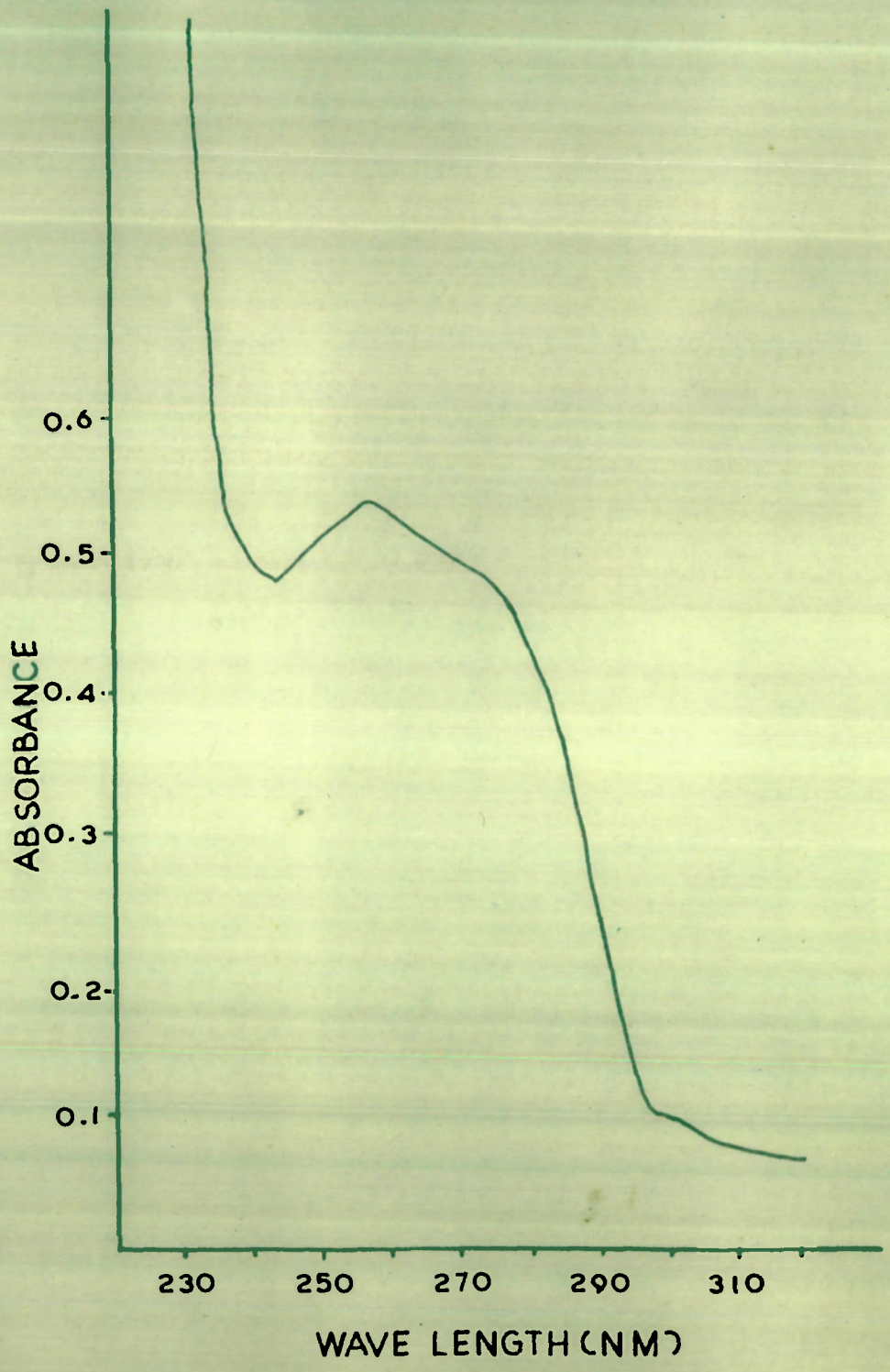

Fig. 23

Fig. 24. Ultracentrifugation Schlieren pattern of purified *Solanum amotapense* mosaic virus. Sedimentation is from left to right, ultracentrifuge run at 20°C and 17000 rpm (a) photographs taken 4 min after attaining maximum speed. (b), (c) and (d), at intervals of 4 min.

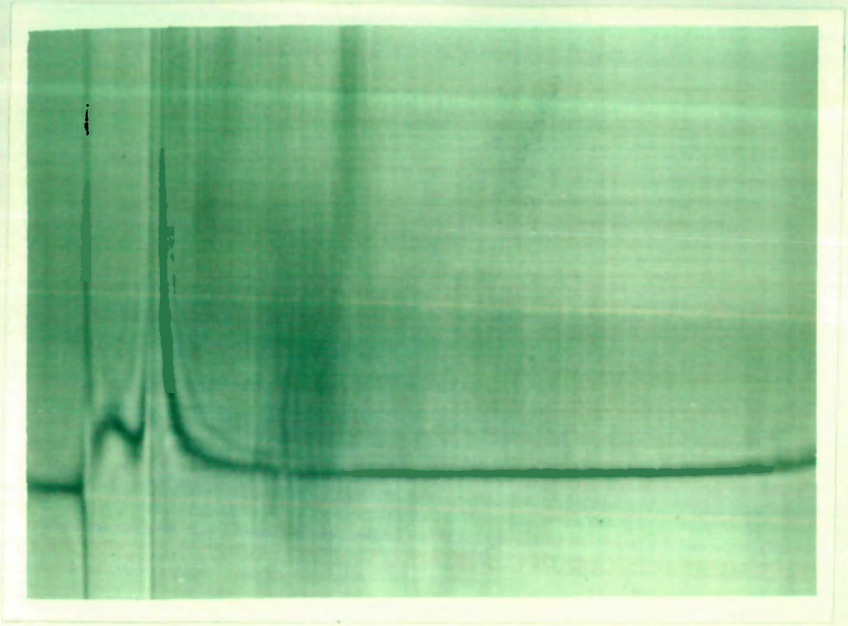

FIG. 24 A

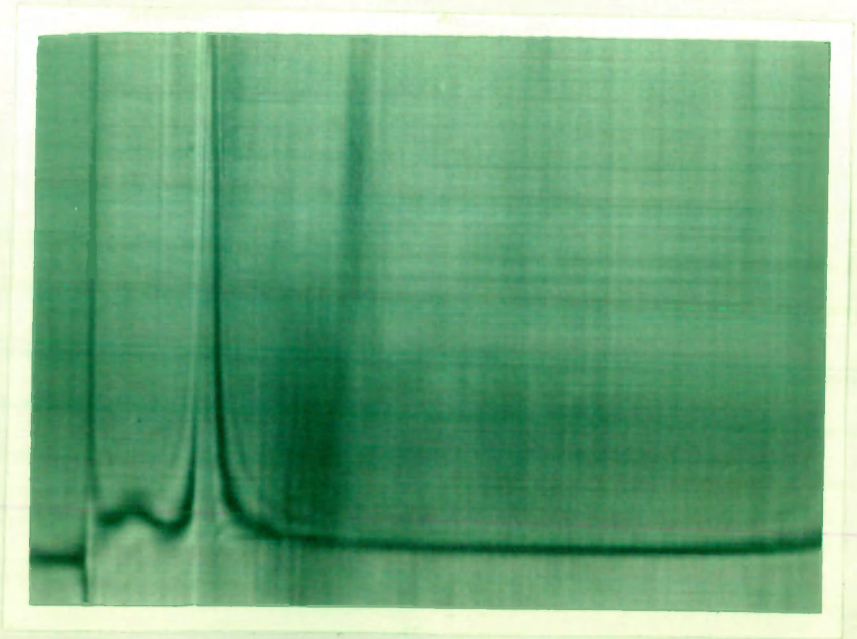

FIG. 24 B

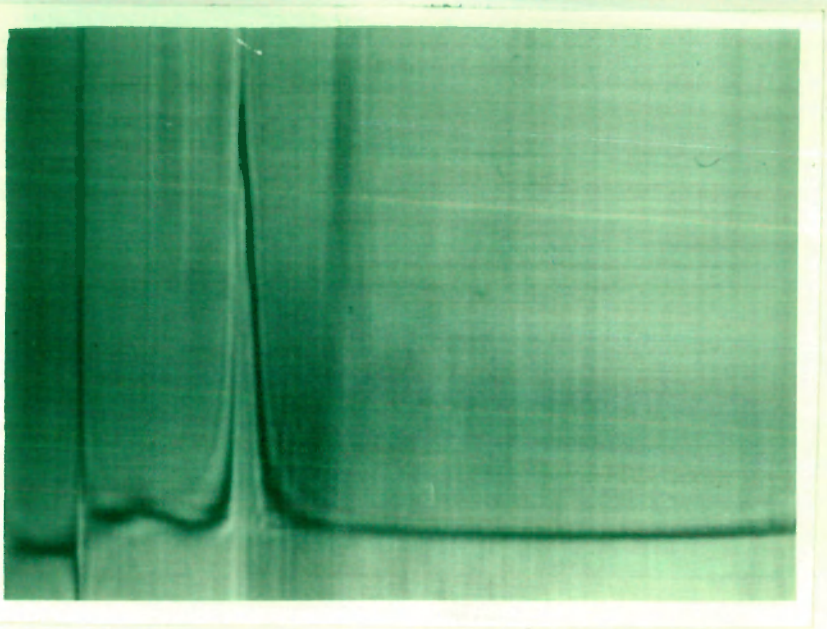

FIG. 24 C

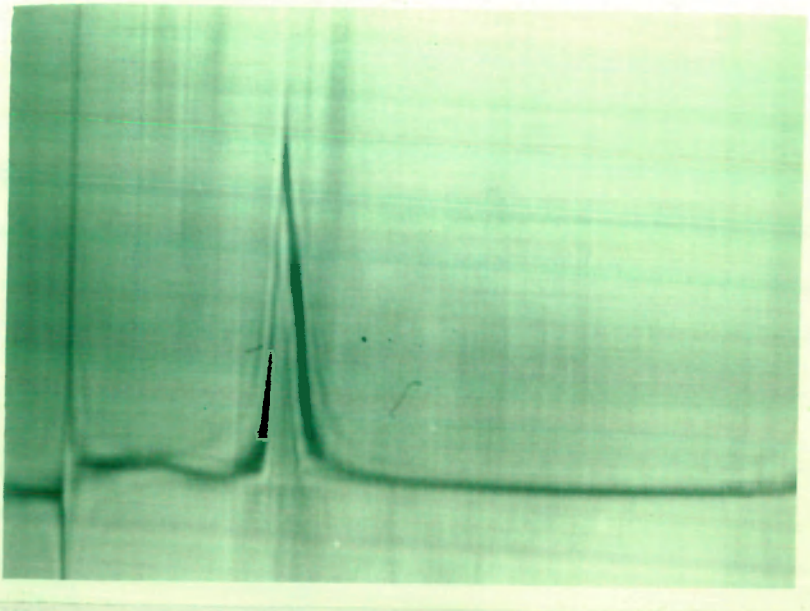

FIG. 24 D

Fig. 25. Electron micrograph of Solanum amotapense mosaic virus from leaf dip preparation. Magnification X 13500.

Fig. 26. Electron micrograph of Solanum amotapense mosaic virus strained with 2 per cent PTA pH 6.5, Magnification x 36000.

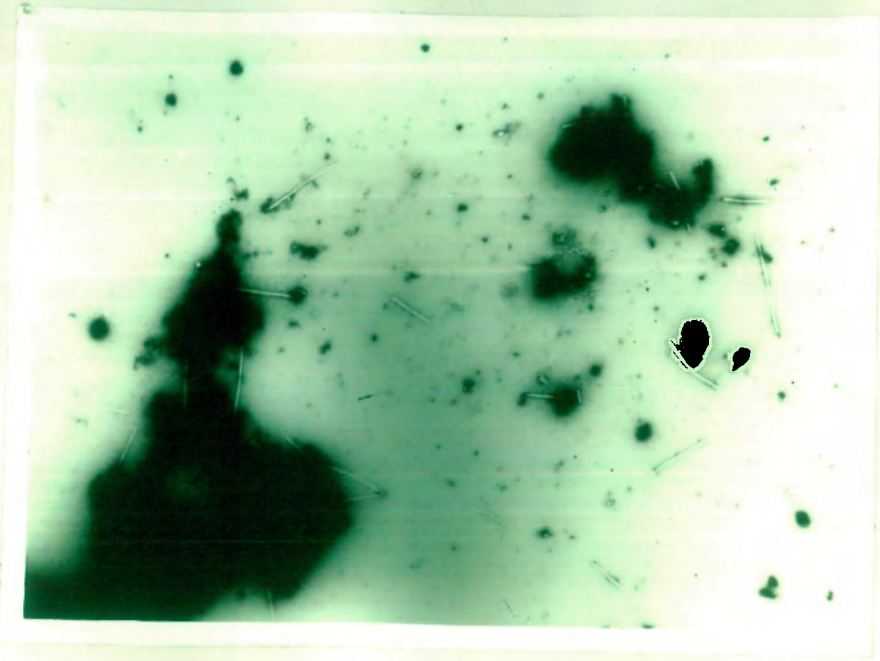

FIG. 25

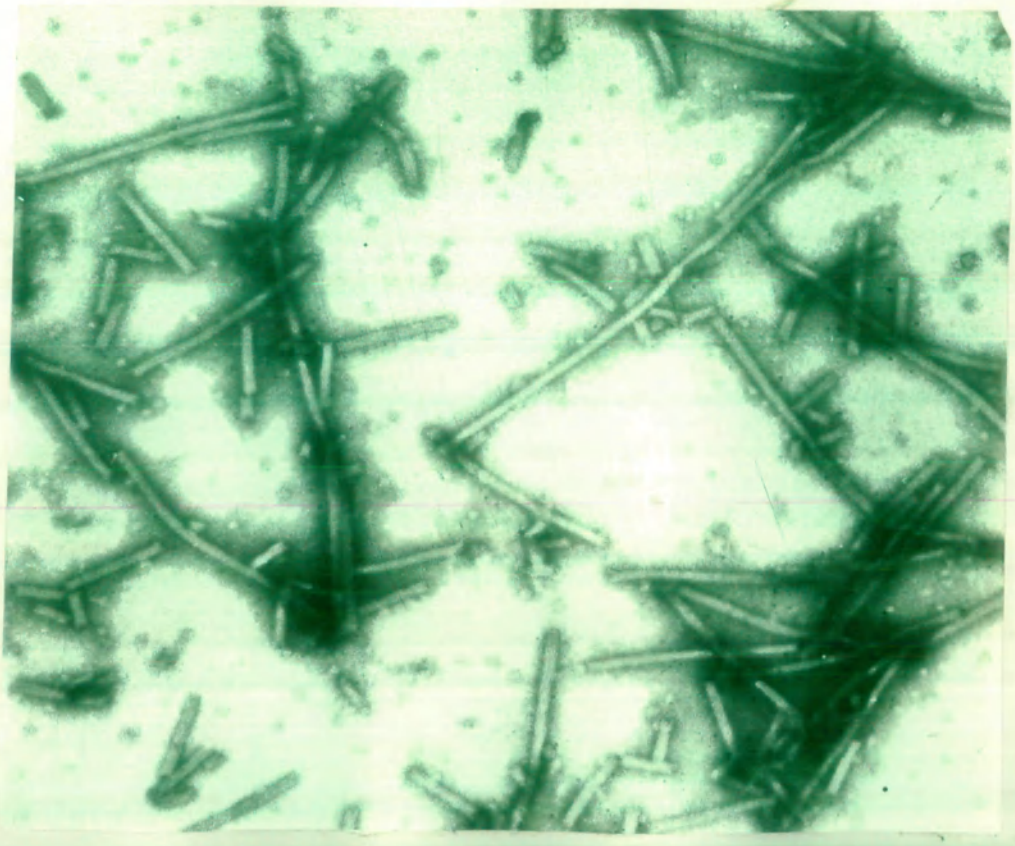

FIG. 26

Fig. 27. Solanum amotapense mosaic virus antiserum (central well) with (clockwise) antigen healthy sap, saline, distilled water and sap from infected plant.

Fig. 28. Agar-gel diffusion test of Solanum amotapense mosaic virus (central well) with (clockwise) antisera against SaMV, cucumber green mottle mosaic virus, pumpkin mosaic virus and normal serum.

Fig. 29. Agar-gel diffusion test of Solanum amotapense mosaic virus (central well) with(clockwise) antisera against cowpea mosaic virus normal serum and saline.

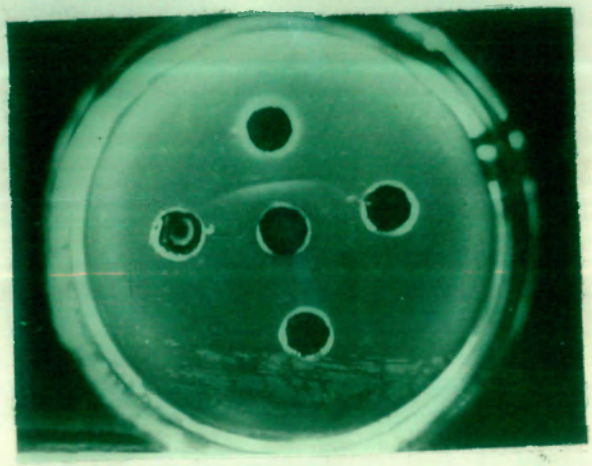

FIG. 27

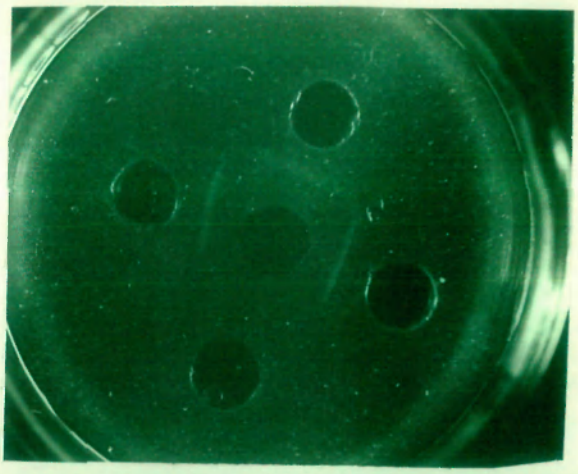

FIG. 28

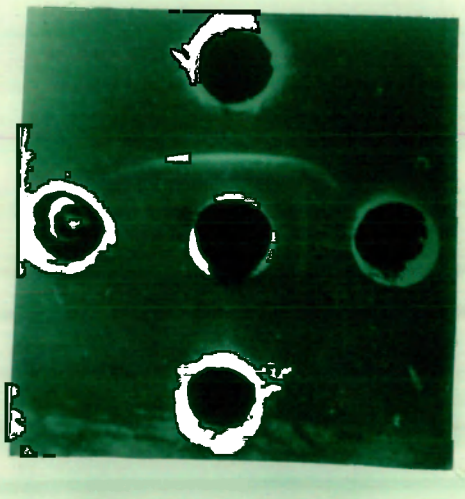

FIG. 29

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